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Determining Attention Deficits In Mouse Models Of Alzheimer's Disease Using Touchscreen Systems

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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Determining Attention Deficits In Mouse Models Of Alzheimer's Disease Using
Touchscreen Systems

(Thesis format: Monograph)

by

Talal Masood

Graduate Program in Neuroscience

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master's of Science

The School of Graduate and Postdoctoral Studies
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Abstract

Behavioural testing in mouse models of Alzheimer's disease (AD) suffers from lack of standardization and reproducibility issues between laboratories. In order to solve this, a touchscreen system has been developed for mice based on the Cambridge Neuropsychological Test Automated Battery (CANTAB). There are several cognitive dysfunctions that occur due to AD, including deficits in attention that can be tested using the touchscreens. In this study, we tested two mouse models of familial AD (5xFAD and 3xTG) with mutations that lead to an accelerated rate of amyloidosis. Both male and female mice were tested at two separate locations in order to test for the reproducibility of results. Mice were tested as they aged (4.5, 7, 10 months) in order to establish when cognitive symptoms first appear, with the 3xTG line showing deficits at 4.5 months and the 5xFAD at 7 months. The results obtained between the two sites were not significantly different for either line.

Keywords

Alzheimer's disease (AD), attention, amyloid-beta ($A\beta$) plaques, amyloid precursor protein (APP), presenilin-1 (PSEN1), tau neurofibrillary tangles (NFTs), familial Alzheimer's disease (FAD), 3xTG, 5xFAD, 5-choice serial reaction time task (5-CSRTT), touchscreen

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1 Introduction

1.1 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting 35 million people worldwide (Organization, 2012). It is the most common cause of dementia, accounting for 60-70% of all cases (Sy et al., 2011). AD affects 1 in 11 people over the age of 65 in Canada and these numbers are expected to triple by the year 2050 (Herbert et al., 2014).

In ancient times, physicians from the Roman and Greek empires associated old age with increased susceptibility to dementia (Berchtold and Cotman, 1998). In 1901, the German physician and psychiatrist Alois Alzheimer identified the first case of AD in a fifty-year old patient (Greenson, 1987). For most of the 20th century, it was difficult to distinguish between dementia and AD, and the diagnosis of AD was reserved for patients exhibiting symptoms between the ages of 45 and 65. In 1977, it was concluded that individuals of any age that displayed the common characteristic symptoms, disease course and neuropathology should be diagnosed with AD, independent of age (Boller and Forbes, 1998).

The earliest symptoms of AD include short-term memory loss followed by difficulty with language, disorientation, and a variety of behavioural changes in motivation and mood (Burns and Iliffe, 2009). As the disease progresses, there is more severe cognitive decline, most notably episodic memory, as well as changes in sleep patterns and deficits in motor functions (McDonald and Overmier, 1998). In addition, there is severe decline

in executive functions; including working memory, sustained attention, task flexibility, problem solving and reasoning (Monsell, 2003).

The pattern of cognitive decline in AD is believed to be the result of neuronal lesions that may have been caused by amyloid-beta ($A\beta$) plaques and tau neurofibrillary tangles (NFTs), leading to synaptic loss, and neuronal cell death (Gotz et al., 2004). In addition, patients suffering with AD also have vascular lesions. It has been suggested that vascular risk factors lead to the dysfunction of the blood-brain barrier and decreasing blood flow, hence reducing the clearance of $A\beta$, leading to a cascade of events preceding dementia (Zlokovic, 2011). Nevertheless, the presence of $A\beta$ plaques and tau neurofibrillary tangles (NFT) are considered to be the pathological hallmark of AD (Duyckaerts et al., 2008).

The average life expectancy of individuals diagnosed with AD is eight to ten years with fewer than 4% of people living longer than fourteen years (Molsa et al., 1986, 1995, Zanetti et al., 2009). In 68% of all cases, the disease itself is the underlying cause of death, usually due to pneumonia and dehydration (Molsa et al., 1986, Ganguli et al., 2005).

1.2 Neuropathology of AD

AD is characterized by loss of neurons and synapses in the cerebral cortex and different subcortical regions. It is believed that $A\beta$ plaques and NFTs lead to degeneration of neurons in the parietal and temporal lobes as well as parts of the frontal cortex (Wenk,

2003). Both A β plaques and NFTs are clearly visible under microscopy of brains of people with AD (Tiraboschi et al., 2004).

Amyloid plaques are dense, mostly insoluble deposits of amyloid-beta (A β 40 and A β 42) peptides and cellular material outside of neurons (Tiraboschi et al., 2004). A β peptides are fragments of the larger amyloid precursor protein (APP), a neuronal transmembrane protein critical to neuron survival, growth, and post-injury repair (Priller et al., 2006). APP is cleaved by the activity of three proteolytic enzymes: alpha, beta, and gamma secretases, however only the latter two give rise to the toxic A β peptides (Hartmann et al., 1997). The A β peptides are excised from the larger APP holoprotein through sequential scission by the β -APP cleaving enzyme (BACE) and the gamma secretase enzymatic complex (Yan et al., 1999).

Mutations in presenilin-1 (PSEN1) and presenilin-2 (PSEN2), proteins that form the catalytic domain of the larger gamma secretase complex (De Strooper et al., 1999, Hooper, 2005) lead to AD. Gamma secretase cleaves within the transmembrane domain of APP and produces the C-terminal end of A β (Hartmann et al., 1997). A β peptides are known to aggregate to form soluble A β oligomers in the extracellular environment and these have been shown to be the most toxic form of A β (Zhao et al., 2012). In AD, A β oligomers accumulate and form fibrils which further form clumps around neurons leading to dense insoluble formations known as senile plaques (Ohnishi and Takano, 2004, Tiraboschi et al., 2004). Plaque deposition normally begins in the basal portions of the neocortex, followed by the hippocampus and eventually spreading to subcortical areas over time (Braak and Braak, 1991).

The second hallmark of AD, NFTs, is caused by the abnormal hyperphosphorylation and misfolding of the protein tau (Bouras et al., 1994). Tau is a microtubule-associated protein (MAP) that is a major component of a group of MAPs that stabilize microtubules and induce its own assembly (Maccioni and Cambiazo, 1995). Microtubules act like tracks, guiding nutrients from the cell body to the ends of axons and back. However, under pathological conditions, tau becomes hyperphosphorylated causing it to self-aggregate into paired helical filaments, which eventually aggregate to create the neurofibrillary tangles in the intracellular environment (Berger et al., 2007). The microtubules are the cytoskeleton for axons and their collapse leads to the disintegration of the neuron's transport system (Hernandez and Avila, 2007).

Although amyloid plaques and NFTs are associated with AD, it is still not clear how they give rise to the neurodegeneration associated with the disease (Huang and Mucke, 2012). As discussed in detail in the next section, the amyloid hypothesis points towards the accumulation of A β peptides as the central event triggering neuronal degeneration. Toxic amyloid oligomers disrupt neuronal signaling leading to aberrant calcium ion homeostasis and apoptosis (Yankner et al., 1990). In addition, it has been suggested that long before the development of senile plaques, A β oligomers attach onto the synapse of neurons disrupting cell-to-cell communication (Lacor et al., 2007, Zhao et al., 2012). The tau hypothesis on the other hand, points towards hyperphosphorylated tau protein as the trigger that leads to degeneration of neurons and eventually cell death (Gray et al., 1987). Normally, organelles are transported to and from the cell body of a neuron through the axon and dendrites, which has a skeleton composed of microtubules (Maccioni and Cambiazo, 1995). Microtubules are stabilized by the tau protein, however in AD, tau

becomes hyperphosphorylated and detaches from the microtubules (Iqbal et al., 2005). Thus, the skeleton of the neuron dissociates and the defective tau protein form filaments that eventually generate the NFTs. Without the microtubule skeleton, and with the accumulation of NFTs, neurons degenerate and connections between the neurons are lost. This eventually causes the death of the neuron (Chun and Johnson, 2007, Hernandez and Avila, 2007). NFTs first develop in the hippocampus, which is essential to memory and learning and then spreads to the whole brain in a centrifugal movement causing atrophy. The progression of AD symptoms follows the pattern of NFT lesions (Iqbal et al., 2005).

1.3 Genetic Underpinnings of AD

The cause of most AD cases is unknown except for the cases where genetic mutations have been identified. Hence, AD can be classified as two main sub-types: sporadic onset AD and familial AD (FAD). Only about 1% to 5% of patients afflicted with the disease have the FAD form, whereas the other 95% to 99% have sporadic onset AD (Delacourt et al., 2002). Based on family and twin studies, the genetic heritability of AD range from 49% to 78% and around only 0.1% of familial cases of AD are early onset meaning they occur before the age of 65 (Wilson et al., 2011).

The genetic basis for sporadic onset AD is not well understood. Overall, there are many different risk factors that seem to lead to the sporadic form of AD and the genetic underpinnings that underlie this form of AD are not immediately obvious. The best-known genetic risk factor is the inheritance of the $\epsilon 4$ allele of the apolipoprotein E gene (APOE $\epsilon 4$), and between 40% to 80% of people with AD present at least one APOE $\epsilon 4$

allele (Strittmatter et al., 1993, Mahley et al., 2006). In addition, the APOE ϵ 4 allele triples the risk of AD in heterozygotes and increases it by 15 times in homozygotes (Blennow et al., 2006). Apolipoproteins have been shown to enhance the breakdown of A β , however the APOE ϵ 4 isoform is not very effective at doing so leading to a build-up of A β peptides (Polvikoski et al., 1995). Although the APOE ϵ 4 allele has been shown to increase susceptibility to develop AD, not all individuals with the gene develop the disease, and many individuals without the allele develop AD (Selkoe, 2012). For example, a study conducted in Nigeria showed no relationship between the APOE ϵ 4 and onset of AD (Hall et al., 2006). In addition to the APOE ϵ 4 allele, there are 19 other genetic loci that have been identified to significantly increase the risk of developing AD (Lambert et al., 2013). A meta-analysis of genome-wide association study (GWAS) conducted by Lambert et. al. in 2013 identified 11 of these genetic loci that increase the susceptibility of late-onset AD (Lambert et al., 2013). One of these loci was SORL1, a neuronal APOE receptor involved in regulating APP. SORL1 was found to have reduced expression in AD (Scherzer et al., 2004). Other loci identified include PTK2 β , an enzyme important for calcium-induced regulation of neuronal ion channels and activation of the map kinase pathway; FERMT2, a protein that is a component of extracellular matrix structures required for control of cell shape; and CLU, a protein associated with the clearance of cellular debris and apoptosis (Lev et al., 1995, Jones and Jomary, 2002, Tu et al., 2003). In addition, mutations in the triggering receptor expressed on myeloid cells 2 (TREM2) have also been shown to be associated with a 3-5 times increased risk of AD. It has been suggested that due to the TREM2 mutation, microglia cells are no longer able

to control the amount of A β peptides present in the brain (Guerreiro et al., 2013, Jonsson et al., 2013).

In contrast, FAD is better understood and the genes involved have been identified and isolated. Most cases of FAD can be attributed to missense mutations in at least one the following three genes: APP, PSEN1 or PSEN2 (Waring and Rosenberg, 2008). There have been more than 50 different APP mutations identified, the most common of which is the V717I, which replaces the amino acid valine with the amino acid isoleucine at protein position 717 (Eckman et al., 1997). The PSEN1 gene, found on chromosome 14, is the most common cause of FAD with over 150 different identified mutations. The PSEN1 gene encodes for the presenilin-1 protein part of the gamma secretase complex (De Strooper, 2007). PSEN1 mutations lead to an altered protein that becomes inactive and causes gamma secretase to cleave at a different location releasing the longer toxic peptides of A β (Zahs and Ashe, 2015). These mutations increase the production of toxic A β peptides that are 42 amino acids in length (A β 42), and have been shown to be the main component of senile plaques. However, a recent study showed mice that are homozygous for PSEN1 mutations have complete loss of function of gamma secretase and die during the embryonic stage. Mice carrying more than one PSEN1 mutation had a decreased production of A β , but an increased ratio of A β 42/A β 40 that exacerbated A β deposition (Xia et al., 2015). The ratio of A β 42/A β 40 has been previously proposed to have important ramifications in the onset of plaques observed in AD, rather than the amount of A β 42 alone (Jarrett et al., 1993).

One of the most well known mutations of AD is the Swedish FAD mutation occurring on the APP gene, originally discovered in 1992 (Mullan et al., 1992). Carriers of this

mutation will pass it on to 50% of their offspring who will go on to develop early onset AD. This mutation however is extremely rare, only found in two Swedish families and never in the general population (Mullan et al., 1992). Similarly the Florida FAD mutation was observed in an individual from Florida who had a family history of dementia with an average onset of 53 years. Once the mutation was expressed in cell cultures it led to a three-fold increase in A β 42 (Eckman et al., 1997). The London FAD mutation was discovered in 1991 in England and was the first described mutation of APP. This missense mutation also increases the levels of A β 42 in many cell types that leads to early onset AD (57 years of age approx.) (Goate et al., 1991).

On the other hand NFTs, the secondary hallmark of AD, caused by the accumulation of hyperphosphorylated tau protein, lack genetic evidence linking any FAD tau mutations to the onset of the disease (Selkoe, 2011). As a matter of fact, disorders involving a tau gene mutation have shown that the development of NFTs alone does not lead to the development of A β plaques. Hence, it has been suggested that A β peptides can lead to the development of NFTs, but tangles do not lead to the development of A β peptides (Selkoe, 2011).

1.4 The Amyloid Hypothesis of AD

The amyloid hypothesis was first introduced in 1991 and builds on the knowledge of the genetics of FAD postulating that accumulation of amyloid plaques are the fundamental cause for the disease (Hardy and Allsop, 1991). It postulates that A β , in a variety of forms, triggers a cascade of events that harm synapses and neurons, producing the

pathological hallmarks of plaques, NFTs, synapse loss and neurodegeneration, that leads to dementia (Hardy and Selkoe, 2002). One aspect of the hypothesis suggests that there is a build-up of soluble A β oligomers due to the activity of β -secretase and gamma secretase on APP, which leads to the insoluble senile plaques (Yan et al., 1999). Some researchers have postulated that toxic A β oligomers, referred to as amyloid-derived diffusible ligands (ADDLs), may be the primary pathogenic form of A β . These ADDLs bind to neuronal surface receptors and alter the structure of the synapse disrupting cell-to-cell communication (Lambert et al., 1998, Lacor et al., 2007). The hypothesis is supported by the location of the APP gene on chromosome 21. Individuals with an extra copy of this chromosome (trisomy 21 – down syndrome) universally exhibit AD by the age of 40 (Lott and Head, 2005).

1.5 The Cholinergic Hypothesis of AD

The cholinergic hypothesis is the oldest postulation of AD. It proposes that AD is caused by reduced synthesis of the neurotransmitter acetylcholine (ACh) due to substantial deficits in the enzyme responsible for making ACh, choline acetyltransferase (ChAT) (Bowen et al., 1976, Davies and Maloney, 1976, Perry et al., 1977). Subsequent discoveries of reduced choline uptake, ACh release, and loss of cholinergic neurons in the basal forebrain, confirmed a substantial presynaptic cholinergic deficit in AD (Whitehouse et al., 1982, Rylett et al., 1983, Nilsson et al., 1986). In addition, at the time, research also showed the importance of ACh for cognitive function, such as learning and memory (Drachman and Leavitt, 1974). Further support for the hypothesis was provided by studies showing strong correlations between the deterioration of the basal forebrain

cholinergic system and the occurrence of senile A β plaques (Perry et al., 1978, Wilcock et al., 1982). However, during the last 20 years, the cholinergic hypothesis has not maintained widespread support, mainly because pharmacological interventions intended to treat acetylcholine deficiency, seen in AD, have not been very effective (Francis et al., 1999).

1.6 Current Pharmacological Interventions for AD

There is no cure for AD, and current pharmacological treatments help delay symptoms and maintain cognition for a short period of time. Despite considerable advances in knowledge regarding the pathogenesis of AD, no concrete treatments have been introduced over the past 25 years (Schneider et al., 2014). There are only five drugs that have been marketed for the treatment of AD: four acetylcholinesterase inhibitors (tacrine, rivastigmine, donepezil and galantamine), and the NMDA receptor antagonist memantine (Schneider et al., 2014). There is a reduction in the release of ACh in AD (Perry et al., 1977). Acetylcholinesterase inhibitors block the activity of acetylcholinesterase, an enzyme found in the synaptic cleft responsible for breaking down ACh, thereby increasing the concentration of this neurotransmitter in the brain (Massoulie et al., 1993). Evidence suggests that the acetylcholinesterase inhibitors approved for treatment seem to have efficacy only in mild to moderate AD and not during the advanced stages of the disease (Birks, 2006, Schneider et al., 2014). Therefore, these drugs are only beneficial in temporarily delaying the onset of cognitive deficits associated with AD.

A review published in 2008 identified 100 AD drug development failures in clinical trials (Becker et al., 2008). In the last two decades (since 1991), the focus of AD drug development has been on the amyloid hypothesis and the toxic A β found in the brain. The majority of drugs that were developed for AD attempted to disrupt the activity of the proteases, β -secretase and gamma secretase, however none have been successful in clinical trials (Becker et al., 2008). Although in theory the amyloid drugs seem simple, either by reducing its production through inactivating the enzymes that produce it or by increasing its clearance out of the brain, it has been quite complex to implement it in practice. One reason for the failure of this approach may be due to differing toxicities of various A β components. For example, oligomers have been found to be toxic at the synapse and A β fibrils from plaques may be pro-inflammatory leading neurotoxicity in their local environment (Mucke and Selkoe, 2012). Hence, it is unclear whether inhibition of A β fibril aggregation, inhibition of APP secretases, or antibodies against the different forms of A β are valid therapeutic strategies (Schneider et al., 2014).

1.7 Development of AD Mouse Models

As with other slowly progressive diseases, preventing AD depends on understanding the early steps in its pathogenesis. For this reason, considerable time and efforts has been invested in the development of animal models for AD. Although it accounts for only a small portion of AD patients, identifying gene mutations involved with the FAD form of the disease greatly enhanced Alzheimer's research (Selkoe, 2012). Researchers were able to create transgenic mice that had the FAD mutations in APP and presenilins that lead to

the expression of A β plaques similar to those seen in humans (Gotz et al., 2004, Schnabel, 2011).

One of the first AD mouse models generated was the Tg2576 that had the APP^{SWE} FAD mutation (Hsiao et al., 1996). The Tg2576 mice showed deficits in spatial working memory at 5 months of age in one study, however different methodologies used in several other reports failed to reproduce these results (Hsiao et al., 1996, Arendash et al., 2004). Studies have also shown the Tg2576 mice to have deficits in recognition memory at 12 months of age (Oules et al., 2012).

Another important mouse model of AD was the APP/PS1 mice that had both the APP^{SWE} mutation as well as a mutation on PSEN1 that lead to A β plaques (Holcomb et al., 1998). The first reported deficits in spatial working memory were 6 months of age in the APP/PS1 mice, however these were not robust and other reports have failed to reproduce these data (Holcomb et al., 1999, Arendash et al., 2001a). Consistent deficits in working memory have been reported in the APP/PS1 mice at 15 months of age and deficits in recognition memory at 12 months of age (Arendash et al., 2001b, Mori et al., 2013). Hence, most of the early mouse models of AD focused on developing the A β plaques pathology and demonstrated robust cognitive deficits in the later stages of the animal's life. In this thesis we will focus on two models of AD described below that either present plaques and NFTs or have high rates of amyloidosis (aggregation of A β) and plaque formation.

1.8 The 3xTG Mouse Model of AD

In 2003, Oddo et al. developed a mouse model of AD that exhibited both A β plaques as well as tau NFTs called the triple transgenic mouse (3xTG). These mice possess the Swedish FAD mutation of APP (APP^{SWE}) as well as a mutation on the presenilin-1 (PSEN1^{M146V}). In addition, the 3xTG mouse line also has a point mutation on a tau transgene (tau^{P301L}) that gives rise to hyperphosphorylated tau protein leading to the development of NFTs (Oddo et al., 2003b). The 3xTG mouse line was generated by microinjecting the human APP_{SWE} and tau_{P301L} transgenes into the germline of PS1_{M146V} knock-in mice. The result was a mouse model that expressed both plaques and tangles that increased in severity with age (Oddo et al., 2003b). 3xTG mice express A β plaques and NFTs in analogous brain regions to that seen in human AD patients, and progression of pathology also follows similar patterns to that seen in humans, with plaque deposition preceding the development of visible NFTs (Oddo et al., 2003b, Sy et al., 2011).

The 3xTG mice show no A β plaques or NFTs at two months of age (Oddo et al., 2003b). However, at this age, other researchers have shown the 3xTG mice to have axonal myelin sheath deterioration in the Schaffer Collateral pathway projecting from the hippocampus (Desai et al., 2009). The appearance of A β pathology occurs first in the neocortex at four months of age, however most of the A β is in its soluble form at this stage and has not started to develop into plaques. At 6 months of age, intraneuronal A β is detectable in both the amygdala and hippocampal neurons (Oddo et al., 2006a). At approximately 9 months of age, there is a decrease in intraneuronal A β that is coincident with the development of extracellular A β plaques in the cortex. These extracellular plaques increase in both size and number exponentially with age and by 12-15 months the 3xTG mice show significant

A β plaques in both the hippocampus and cortex (Oddo et al., 2003b, Oddo et al., 2006a, Sy et al., 2011).

Similarly, AD-like tau pathology also progresses with age in the 3xTG mice. Hyperphosphorylated tau protein can first be detected in the CA1 pyramidal neurons of the hippocampus at 6 months of age (Oddo et al., 2003a). Levels of abnormally phosphorylated tau continue to increase in the hippocampus and by 9 months of age are also detectable in the cortex. By the time mice are 10 months of age, hyperphosphorylated tau aggregates to form NFTs in both the cortex and hippocampus. The density of these NFTs continues to increase exponentially as mice age (Oddo et al., 2003a, Oddo et al., 2003b).

The 3xTG mice have been shown to have cognitive deficits in various behavioural studies. The 3xTG mice were tested at 2 months of age on the Morris Water maze task but showed no deficits (Clinton et al., 2007). However, in a separate study conducted using the 8-arm radial maze task to test for working memory, the 3xTG mice did show deficits starting at 2 months and onwards until the age of 15 months (Stevens and Brown, 2015). The first deficits observed in associative learning occur between the age of 3 and 5 months (Clinton et al., 2007). These are then followed by deficits in spatial working memory using the Morris Water Maze task at 6 months of age (Billings et al., 2005, Nelson et al., 2007). Deficits in recognition memory have been observed between 9-11 months of age (Billings et al., 2005, Clinton et al., 2007, Nelson et al., 2007). Also, impairments in reference memory were observed in the 3xTG mice using the Barnes Maze task at 12 months of age (Nelson et al., 2007). In addition, as discussed in greater

detail in the next section, 3xTG males showed deficits in sustained attention when tested using touchscreen systems at 10 months of age (Romberg et al., 2011).

Interestingly, a recent study showed that short-term stress induced a decrease in the number of synaptic dendritic spines and increased the levels of A β in 3xTG mice, hence accelerating amyloid pathology (Baglietto-Vargas et al., 2015). Additionally, it has been shown that cholinergic modulation of hippocampal synaptic plasticity is impaired due to the PSEN1 mutation in 3xTG mice (Wang et al., 2009). In summary, the neuropathology of AD-like plaques and tangles as well as the AD-like cognitive deficits strengthens the face validity of the 3xTG as a mouse model of AD.

1.9 The 5xFAD Mouse Model of AD

Most mouse models of AD, including the above mentioned 3xTG mice, develop neuropathological and significant behavioural deficits in the latter stages of life. Although this increases the face validity of these mouse models since they recapitulate the late onset of AD as seen in humans, it proposes other technical disadvantages such as high housing costs for animal facilities, and long delays between planning and performing experiments (Bilkei-Gorzo, 2014). For these reasons, a new transgenic mouse line was developed by Oakley et al. in 2006 bearing five FAD mutations called the 5xFAD mice, that developed AD-like pathology at a much younger age (Oakley et al., 2006). The 5xFAD mice have three FAD mutations on the APP transgene with the Swedish (APP_{SWE}), Florida (APP_{FL}) and London (APP_{LON}) mutations, and two FAD mutations on

the presenilin-1 transgene (M146L and L286V). These mutations were introduced into mice using the neuron specific Thy1 promoter (Oakley et al., 2006).

Together, all five of these FAD mutations lead to an accelerated rate of intracellular amyloidosis starting at just 1.5 months of age and extracellular A β plaques are present at 2 months in this mouse model. A β plaques first appear in layer 5 of the cortex and the subiculum at 2 months of age, and spread rapidly to fill the rest of the cortex, subiculum and hippocampus by 6 months (Oakley et al., 2006). Due to this rapid amyloidosis, by 9 months of age, the 5xFAD mice have significant synaptic degeneration and neuron loss (Oakley et al., 2006, Bilkei-Gorzo, 2014).

Multiple studies have observed progressive cognitive deficits with age in the 5xFAD mice. This model shows deficits in spatial working memory using the Morris Water Maze by 3 months of age (Ohno et al., 2006, Urano and Tohda, 2010). In a study used to test for hippocampal-dependent memory deficits using the olfactory tubing maze, 5xFAD mice showed deficits beginning at 4 months of age and the deficits increased significantly by 6 months (Girard et al., 2014). 5xFAD mice also showed hippocampal-dependent spatial working memory deficits when tested using the Y-maze task at 4 months of age but not at an age of 2 months (Oakley et al., 2006). Additionally, 5xFAD mice showed impairments in associative learning, in fear conditioning, as well as deficits in working memory using the Y-maze task (Oakley et al., 2006, Ohno et al., 2006). As seen in 3xTG mice, cognitive deficits in recognition memory are observed last in the 5xFAD mice - at 10 months of age (Tohda et al., 2012). In summary, although 5xFAD mice do not exhibit tau NFT pathology like 3xTG mice, they do have an accelerated rate of amyloidosis

leading to extensive A β plaques and neurodegeneration that contributes to the various AD-like cognitive deficits observed.

1.10 Attention Deficits in AD

Attention is the first non-memory domain affected in AD, before deficits in visuo-spatial function and language. It is believed that attention deficits are responsible for the difficulties with daily living for patients in the early stages of AD (Bracco et al., 2014). Lesion studies in mice have revealed multiple forebrain regions, such as the medial prefrontal cortex, anterior cingulate cortex and the parietal cortex, to be essential in choice accuracy for mice (Muir et al., 1996). Brain correlates for attention in humans has been shown to be in similar regions, mainly the frontal and parietal cortices (Pepeu et al., 2013). In terms of neurotransmitter systems involved in attentional control, major findings over a 20-year period, have shown selective lesions in the monoaminergic (noradrenergic, dopaminergic and serotonergic) and cholinergic systems to cause the greatest deficits (Robbins, 1997, Pepeu et al., 2013). Since arousal and attention depend on activation of the forebrain cholinergic system, it is to be expected that the loss of cholinergic neurons occurring in AD may lead to impairment of the attentional processes (Pepeu et al., 2013). Improvement of attention following cholinesterase inhibitors, such as donepezil, administration has been demonstrated in mouse models of AD (Romberg et al., 2011). In addition, cholinesterase inhibitors have been shown to improve global cognitive measures in AD and attenuate the neuropsychiatric symptoms through a primary effect on the cholinergic neurons involved in regulating attention (McGuinness et al., 2010, Pepeu et al., 2013).

1.11 CANTAB Touchscreen Systems

Despite over thirty years of research on AD and numerous potential treatments that were tested in clinical trials, there have been no drugs that have been able to halt or reverse the progression of the disease (Selkoe, 2012, Schneider et al., 2014). There have been many pharmacological interventions that have shown promise in pre-clinical animal studies of AD, but have failed to translate the same results in the clinic (Schneider et al., 2014). One critical issue that has been identified for this lack of translatability is the difference between tests of cognition used for mice in pre-clinical research and those used in humans with AD (Romberg et al., 2013a). The Cambridge Neuropsychological Test Automated Battery (CANTAB) has been used to assess cognitive dysfunctions in neuropsychiatric and neurodegenerative disorders including AD and mild cognitive impairment (Junkkila et al., 2012). Based on the CANTAB battery, a touchscreen system has been developed by Tim Bussey, Lisa Saksida and colleagues, to make behavioural cognitive tests in rodents more standardized, automated and increase the translational potential of research in mouse models (Bussey et al., 2008).

The first advantage of using the touchscreen systems to test for cognitive deficits in mice is that they are automated. Having an automated behavioural test reduces the amount of labour required and allows researchers to test multiple mice simultaneously. Moreover, an automated behavioural test such as these touchscreens allows researchers to profoundly reduce the interaction between animals and the experimenter eliminating confounds that this interference might entail during testing (Bussey et al., 2012). Human

interference during behavioural testing can have huge effects, not only by introducing variability in the way the test is run, but also by potential confounds in the way each experimenter handles the animals, or even the sight and smell of the experimenter (Wahlsten et al., 2003). In addition, since a computer controls the touchscreen systems, many parameters including stimulus presentation and delays are identical every time for each animal and all measures of the test are then collected by the computer making it possible to collect data down to the exact millisecond (Bussey et al., 2012). The use of a computer-controlled system also allows for the collection of multiple parameters at once such as accuracy, omissions, and latencies to name a few. Overall, due to their automation, the touchscreen systems might be more reliable than non-automated behavioural tests such as mazes.

The second advantage of using touchscreen systems is that they cause low stress to the animals. It is important to avoid unnecessary stress when testing for cognitive deficits in diseases such as AD. The touchscreen systems used in this study are reward based, rather than aversive thus reducing the amount of stress an animal might encounter (Bussey et al., 2012).

Finally, the main advantage of using touchscreen systems is their translatability. By creating the same tasks for mice as the CANTAB, researchers can test for the same cognitive domains that are tested in humans with AD and other mild cognitive disorders (Bussey et al., 2012). Currently the tests used in animals, such as the Morris water maze, are usually nothing like those we use in clinical populations; i.e. humans who are old and might be suffering from AD do not have their working memory tested in a swimming pool with a hidden platform like mice tested in the Morris water maze task. This creates a

huge disconnect when promising results found in animal research do not translate into the same outcomes in the clinic (Bussey et al., 2012). By using the touchscreen systems that are as similar as possible to human tests, this study aims to increase the face validity of behavioural testing and increase the translational effectiveness of future studies in the clinic.

The touchscreen systems can be used in various different rodent tasks to test for deficits in learning and memory, executive function, working memory, and pattern separation. For learning and memory, there are a number of different tasks that can be used on the touchscreen systems. The visual discrimination (PD) task is relatively simple and used to test whether animals can distinguish between two different stimuli with one being the conditioned stimulus (CS^+) that is rewarded compared to the second stimulus (CS^-) (Horner et al., 2013). In the paired-associate learning (PAL) task, the correct stimulus is identified by the combination of the stimulus itself and spatial location on the screen (Horner et al., 2013). The visuomotor conditional learning (VMCL) task requires the animal to identify the conditional visual stimulus that is presented and answer correctly (left or right) (Horner et al., 2013).

For testing executive functions, Bussey and his colleagues have described several other tasks that can be utilized on the touchscreen systems. First, extinction learning can be used to assess the rate and extent of curtailing a stimulus that was previously associated with a reward but no longer is (Mar et al., 2013). Secondly, reversal learning can be testing using the PD task by switching the CS^+ stimulus with the CS^- stimulus and measuring the rate it takes an animal to extinguish the initial task and learn the new one (Mar et al., 2013).

Lastly, the 5-Choice Serial Reaction Time Task (5-CSRTT) can be used in order to test for the ability of an animal to selectively detect and respond to spatially unpredictable visual stimuli (Mar et al., 2013). The original development of the 5-CSRTT was based on the continuous performance test designed by Rosvold and Mirsky in a nine-hole operant chamber, used to test sustained and divided attention (Beck et al., 1956). Many early attempts to examine attention control in animals used this nine-hole box (Carli et al., 1983). Rodents had to monitor the occurrence of a brief target stimulus (light) in one of five holes at the back of the box, detected by a nose-poke response monitored by infrared photocell beams, and correct responses rewarded by a food pellet in the magazine tray at the rear of the chamber (Robbins, 2002). The task was essentially designed to test the ability of the rodent to sustain spatial attention divided amongst a number of locations over a number of trials and this is measured by the accuracy of the total number of correct trials (Robbins, 2002). Difficulty in the 5-CSRTT can be varied by adjusting the brightness of the stimuli, adding distracting stimuli such as bursts of white noise or presenting visual stimuli in locations not associated with the test (Robbins, 2002). The 5-CSRTT used in the present study utilized the Bussey touchscreen chambers to present rodents with 5 spatial locations on a screen in order to test attentional demand at various stimulus lengths (Mar et al., 2013).

1.12 Objectives of this Study

Hypothesis: Attention deficits are common behavioral abnormalities in mouse models of AD. The 3xTG and 5xFAD mouse models will both show attentional deficits using the 5-

CSRTT. The appearance of the phenotype will vary depending on the severity of AD pathology for each model.

Rationale: Attentional control, part of the overall executive functions, declines during the early stages of AD (Baddeley et al., 2001). Like memory, attention is controlled by multiple cortical and subcortical regions in the brain (Duncan, 2006). Although AD is most commonly known as a memory disorder due to the loss in episodic memory, deficits in sustained attention and executive functions occur earlier in the disease and are usually overlooked by patients (Artero and Ritchie, 2003). The deficits in executive control (including attention) precede impairments in language, spatial and long-term memory (Bentley et al., 2008). Hence, understanding the nature of attention deficits that occur early in AD may provide insights into the mechanism and lead to potential pharmaceutical interventions that can be applied before the disease further advances.

Aim 1: The first aim was to test the 3xTG and 5xFAD mouse models of AD for attention deficits as they age, using the 5-CSRTT. Multiple cognitive domains are compromised in AD. Deficits in executive functioning and sustained attention occur during the early stages of AD (Romberg et al., 2013a). Understanding the characteristics of attention deficits in AD can help detect the disease during its early stages before it progresses. The 3xTG and 5xFAD mouse lines were selected for this study as they have the fastest progression of AD-like pathology and cognitive symptoms and would be ideal candidates for future studies in order to test for potential therapeutic interventions. In this study, we proposed to determine if and when each mouse line develops deficits in sustained attention. Male 3xTG mice were previously tested using the 5-CSRTT by Romberg et. al. in 2011 and were shown to have deficits at 10 months of age (Romberg et al., 2011).

However, as mentioned above, the 3xTG mice develop plaques and tangles earlier and have shown to have cognitive deficits between 3-5 months of age (Clinton et al., 2007). In addition, the 5xFAD mice have been shown to have A β plaques at 2 months of age and working memory deficits present at 3 months of age (Oakley et al., 2006, Ohno et al., 2006, Urano and Tohda, 2010). Hence, in this longitudinal study, both mouse lines were tested multiple times on the 5-CSRTT as they aged (4.5, 7, 10 months) in order determine when attention deficits first appear and whether these deficits get progressively worse with age.

Aim 2: The second aim of this study was to determine whether data are reproducible when the 5-CSRTT is run at two different sites. Traditional cognitive behavioural testing in mouse models is subject to extreme variation (Wahlsten et al., 2003). There are multiple reasons as to why this occurs including the use of distinct protocols used by different labs, and experimenter error or bias due to lack of automation (Landis et al., 2012, Button et al., 2013). The touchscreen systems used in this study were designed to overcome these impediments. Reproducibility of data is key in cementing the touchscreen systems as a valid behavioural paradigm in order to determine cognitive deficits in mice. However, this has not yet been tested systematically. Thus, this study tested both the 3xTG and 5xFAD mice at the University of Western Ontario and at Dr. Boyer Winters' laboratory at the University of Guelph simultaneously (experiments conducted by Daniel Palmer).

Aim 3: The last aim of this study was to determine whether there are sex differences in the 3xTG and 5xFAD mouse models of AD. For humans, almost two-thirds of individuals affected with the disease are women, suggesting females are

disproportionately affected by AD (Bennett, 2007). The primary explanation for this is due to the fact that women live longer and AD occurs during the latter stages of life (Seshadri et al., 1997, Bennett, 2007). However, many age-specific studies conducted have not been able to find any significant conclusions that age is the reason why women have a higher prevalence for AD (Seshadri et al., 1997, Hebert et al., 2001). Hence, there is no evidence that women are more likely to develop AD than men at any given age. Thus, the study conducted here tested both male and female mice for the 3xTG and 5xFAD mice in order to determine whether there is any difference in the onset and progression of cognitive deficits. To the best of my knowledge, this is the first time female mice were tested on the touchscreen systems.

2 Materials and Methods

2.1 3xTG Mice

The 3xTG mice (B6;129-*Psen1*^{tm1Mpm} Tg(APP^{Swe},tau^{P301L})1Lfa) and age-matched control mice (B6129SF2/J) used in this study were all bred at the Jackson Laboratory in Bar Harbor, Maine, and shipped to Canada for behavioural testing. In summary, the 3xTG mice were homozygous for three mutations (PSEN1, APP^{SWE}, and tau^{P301L}) (Oddo et al., 2003b). The wild-type control mice were generated by crossing C57BL/6J females (B6) and 129S1/SvImJ males (129S) in order to create the B6129SF2/J mice.

The mice used in the present study, twelve 3xTG males, twelve 3xTG females, twelve B6129SF2/J wild-type control males and twelve B6129SF2/J wild-type control females, were three months of age at the start of behavioural testing. At this age 3xTG mice exhibit few or no extracellular A β deposits and relatively low levels of hyperphosphorylated tau (Nelson et al., 2007, Hirata-Fukae et al., 2008).

2.2 5xFAD Mice

The 5xFAD mice (B6.Cg-Tg(APP^{Swe}FILon,PSEN1*M146L*L286V)6799Vas/J) and age-matched wild-type controls (B6SJLF1/J) were also bred at the Jackson Laboratory in Bar Harbor, Maine, and shipped to Canada for behavioural testing. The mice used in the present study, twelve 5xFAD males, twelve 5xFAD females, twelve B6SJLF1/J wild-type control males, and twelve B6SJLF1/J wild-type females, were three months of age at the start of behavioural testing. The 5xFAD mice have previously demonstrated to have intraneuronal A β present starting at 1.5 months of age (Oakley et al., 2006).

2.3 Housing and Food Restriction

Mice of the same genotype and sex were housed in groups of 2, in a room with a 12-hour light/dark cycle (lights turn off at 7:00 P.M.). All behavioural testing was conducted during the light phase of the cycle. Mice were maintained on a restricted diet and kept at 85% of adult baseline body weight during the experiment. This was done in order to ensure the animals were motivated to perform the behavioural touchscreen tasks. All mice were put on food restriction one week prior to the start of behavioural testing and the body weights were gradually lowered to 85% of the original weight.

To determine the weight curve of a typical mouse as it ages, mouse weights of wild-type mice at various ages, ranging from pups to adult mice were recorded (**Figure 1**). Based on these weight curves for both male and female mice (**Figure 1**), we determined the ideal 85% body weight for males should be 23.0 grams and females 20.0 grams, at the start of behavioural training. Once body weight was reduced to 85% of baseline for an individual mouse, it was safely maintained throughout the testing period by providing 2.5g of food per day for males and 2.0g of food per day for females (Tekland Chow – Harlan). Mice were weighed every other day in order to ensure maintenance of body weight at 85% of original weight. Water was available *ad libitum* throughout the experiment.

There was a criteria established in the SOP to drop any mouse that does not acquire the ability to perform on the touchscreen systems during training. Data from mice that did not survive or were dropped due to lack of performance was not included in the final figures.

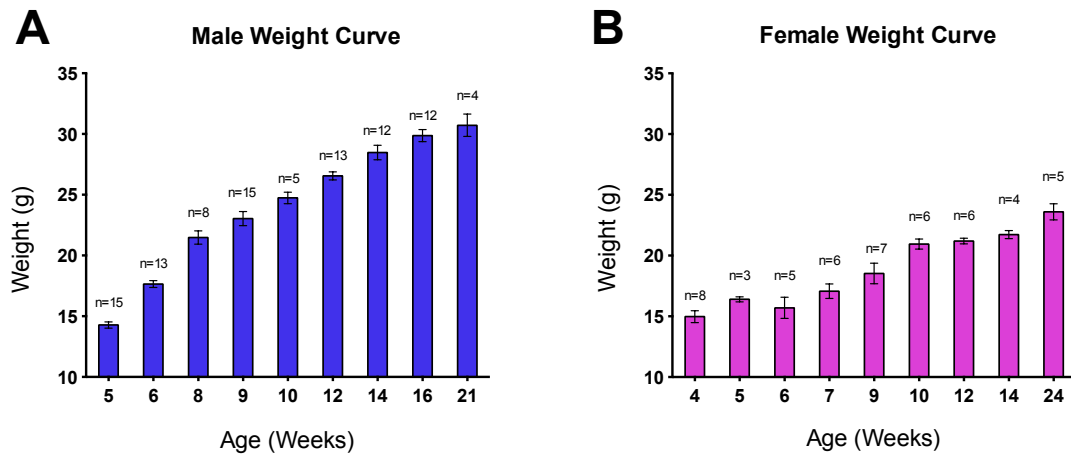


Figure 1. Male and female wild-type weight according to age. Behavioural testing commenced when the mice were 12 weeks of age. Based on these weight curves it was determined the 85% baseline body weight for males was 23.0g and females 20.0g, in order to ensure motivation. (Data are mean \pm SEM).

2.4 Touchscreen Apparatus

Behavioural testing was conducted in the automated Bussey-Saksida Touchscreen System model 81426 (Campden Instruments) for mice (**Figure 2**). The apparatus consisted of a testing chamber housed within a sound and light-attenuating box (40 by 34 by 42 cm). The inner operant chamber consisted of a stainless steel grid floor, black plastic walls, and a clear plastic cover for the top. The operant chambers were fitted with a fan for ventilation and masking of extraneous noise and a reward tray that dispenses liquid reward through a pump. At the other end of the chamber, opposite to the reward tray, was a LCD monitor equipped with an infrared touchscreen controlled system by ABET II Touch software v.3.1 (Lafayette Instruments). A black plastic cover with 5 small response windows (2cm by 2cm) was placed in front of the monitor in order to reduce unintended responses by the tail or other body parts of the mouse (**Figure 2**). The operant chambers were also equipped with a house light and a tone generator. All 5-CSRTT schedules were designed and data were collected using the ABET II software.

2.5 Shaping

Mice were trained to operate the touchscreens by a series of shaping procedures (**Figure 3**) (Mar et al., 2013). All schedules used during the training for the 5-CSRTT were pre-installed by the ABET II software. Please refer to the appendix attached for a more detailed operating procedure for the 5-CSRTT. For the first four days, animals were habituated to the testing chambers using the Habituation schedules. On the first day (Habituation 1), the mice remained in the testing chambers for 10 minutes, the house

lights were off, and there were no touchscreen stimuli or strawberry milkshake reward presented. For the next

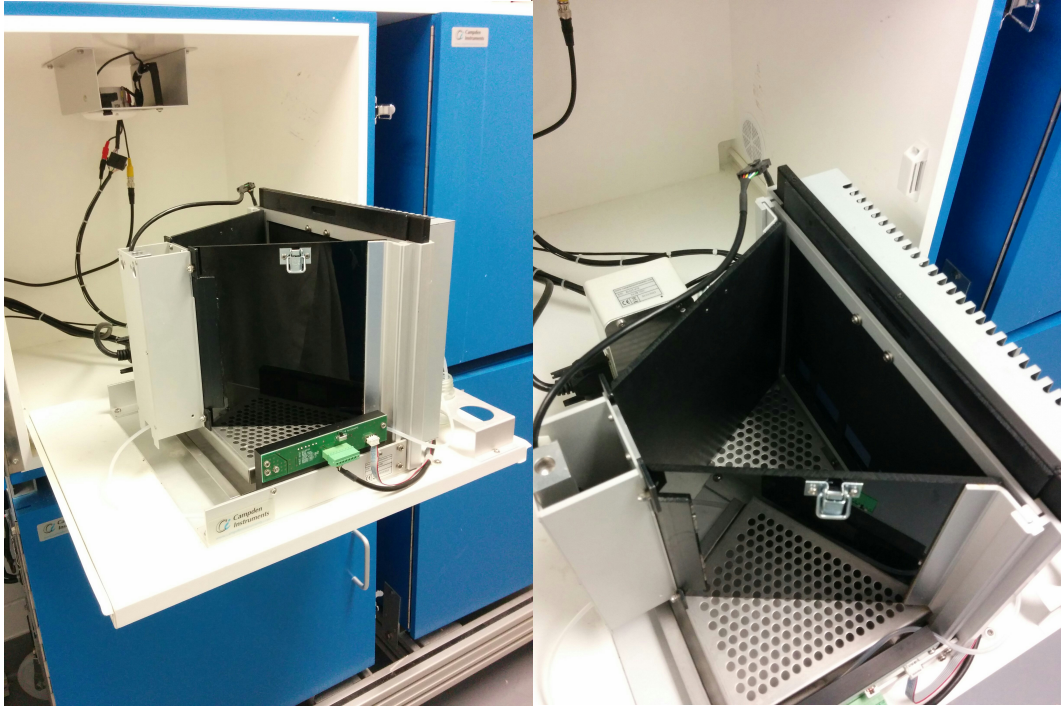


Figure 2: Photograph of the touchscreen chambers. On one side of the chamber is the touchscreen monitor covered by a black plastic mask with 5 small windows (2 cm by 2 cm). On the opposite side of the chamber is the reward tray where strawberry milkshake is dispensed. The entire operant chamber is enclosed within a sound and light-attenuating box.

5 CHOICE SERIAL REACTION TIME TASK (5-CSRTT)

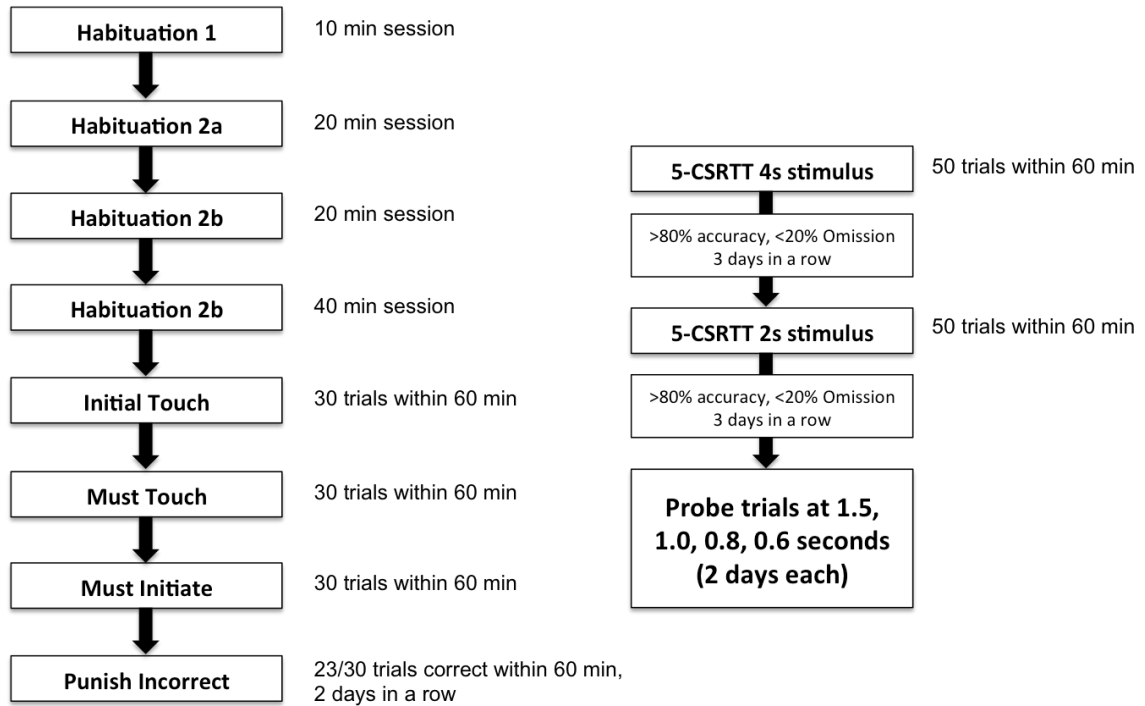


Figure 3: Flow chart of the 5-CSRTT schedules. On the left, all schedules pertaining to the training phase of the touchscreen systems. On the right, training in the 5-CSRTT itself with the 4 seconds, 2 seconds, and probe trial schedules last. Each schedule also includes the criteria required to pass on to the next.

two days (Habituation 2a), the mice were placed into the testing chambers for 20 minutes. During this phase, the reward tray light was turned on and primed with strawberry milkshake (150 μ l) along with a tone. The light remained on until the mouse entered the reward tray and turned back on 10 seconds after the mouse left the tray where more milkshake was provided (7 μ l) along with a tone. During the last day of habituation (Habituation 2b), the mice were placed in the testing chambers for 40 minutes and were subjected to the same schedule as the previous two days. Again, in this phase the mice further strengthened the association of the reward tray light and tone with a small milkshake reward (7 μ l).

Following the habituation, the mice were subjected to the initial touch schedule (one day, 30 trials). A single stimulus (a white square box, 2 cm \times 2 cm) was randomly displayed in 1 of the 5 response windows. After 30 seconds, the stimulus disappeared, the illumination of the reward tray light coinciding with a tone, and the delivery of strawberry milkshake reward (7 μ l). Once the mouse collected the milkshake, the reward tray light extinguished and the next trial commenced after a 5 seconds inter-trial interval (ITI; houselight off, reward tray inactive, no stimulus presented). A response to the stimulus on the screen was rewarded with the delivery of a tone and three times the amount of strawberry milkshake (21 μ l). Training continued on the initial touch schedule until the mice completed 30 trials within 60 minutes.

During the next schedule, called must touch, the stimulus remained on the screen until the mouse touched the correct response window, which resulted in reward of strawberry milkshake (7 μ l), illumination of the tray light, and a tone. The collection of the milkshake triggered the 5 seconds ITI after which the next trial commenced, and a new stimulus was

presented in 1 of the 5 response windows. Each animal continued training until it completed 30 trials within 60 minutes.

The next stage of shaping introduced the animals to the initiation procedure, a schedule called must initiate. At the beginning of each trial, the reward tray was illuminated, and the animal was required to initiate the stimulus delivery by a nose poke into the reward tray. Successful initiation was indicated by the extinction of the tray light and the subsequent display of a stimulus. After touching the stimulus and collecting the reward, the mouse was subjected to the 5 seconds ITI before the illumination of the reward tray light indicated the beginning of the next trial. Once animals readily initiated trials and completed 30 trials within 60 min they moved onto the last shaping phase.

During the last shaping stage, the mouse learns to both initiate and touch the touchscreen stimulus in its correct location. This schedule is similar to the previous must initiate schedule, however, if a mouse touches an incorrect (non-illuminated) location on the screen, the house lights will be turned on for 5 seconds and no reward is given. This is followed by the regular 5 seconds ITI period prior to the start of the next trial. The mice continue to do this phase of shaping until they are able to obtain at least 23 out of 30 trials correct within 60 minutes, on two consecutive days. Once the mice passed the punish incorrect schedule, they went on to train in the 5-CSRTT itself.

2.6 5-CSRTT Training

Mice were trained to respond to brief flashes of light pseudo randomly displayed in one of the five response windows on the touchscreen. Mice were tested 5 days a week, 50

trials or 60 minutes a day. Each trial began with the illumination of the reward tray that elicited a nose poke from the mice. The stimulus was then displayed in one of the response windows pseudo randomly after a 5 to 10 seconds variable delay instead of an immediate presentation of the stimulus as in the previous shaping schedules. If a mouse prematurely touched the screen during this delay, the response was recorded as premature and the mouse was punished with a 5 seconds time out (housetlight on, reward tray inactive). This was followed by a 5 second ITI (housetlight off, reward tray inactive), after which the illumination of the reward tray light signaled the onset of the next trial (Mar et al., 2013). The touchscreen stimulus duration was initially set to 4 seconds, followed by a limited holding period of 5 seconds, during which the animal was still able to respond to the location where the stimulus appeared. Responses during stimulus presence or the limited holding period were recorded either as correct (stimulus window) or incorrect (any other window). A correct choice was rewarded with a tone and the illumination of the tray light, and strawberry milkshake delivery (7 μ l). Reward collection turned the reward tray light off and triggered the 5 seconds ITI. An incorrect response was punished with a 5 seconds time out (housetlight on, reward tray inactive), followed by a 5 seconds ITI (Mar et al., 2013). Failure to respond to any window by the end of the limited holding period was recorded as an omission and punished with a 5 seconds time out, followed by the 5 seconds ITI before the start of the next trial. The mice continued on the 4 seconds stimulus duration until performance was stabilized greater than 80% accuracy, less than 20% omissions for 3 consecutive days. Once the mice had passed these criteria, the stimulus duration was reduced to 2 seconds. After reaching the same criterion with the 2

seconds stimuli (<80% accuracy, >20% omission, for 3 consecutive days), the mice were tested on the four 5-CSRTT probe trial schedules (Mar et al., 2013).

2.7 5-CSRTT Probe Trials

After completing training on the 5-CSRTT 2 seconds stimulus duration, the mice were challenged by reducing the stimulus duration to 1.5, 1.0, 0.8 and 0.6 seconds. At this shorter stimulus duration, the mice required increased attention demand in order to successfully complete the task. The sequence of stimulus duration was presented randomly to each mouse in order to control for order effects. Each mouse performed 2 consecutive days at a given probe trial stimulus duration (1.5s, 1.0s, 0.8s, or 0.6s), and was then moved back onto a 2 seconds stimulus duration for 2 days before they were subjected to the next probe. The mice continued on the probe trials until they had completed all four, taking a total of 14 days.

2.8 Longitudinal Testing

Mice were tested as they aged at three separate time points. The first probe trials were completed when the mice were 4-5 months of age. After the completion of the first set of probe trials, all mice were kept on a maintenance schedule (2 seconds stimulus duration) at least once a week until the commencement of the second set of probe trials that started at 7 months of age. This was to ensure that the mice did not lose the ability to use the touchscreen systems and hence would not require retraining prior to the subsequent 7 and 10-month probe trials. Upon the completion the second set of probe trials, the mice were put back on a weekly 2 seconds maintenance schedule until the commencement of the third set probe trials at 10 months of age.

2.9 Touchscreen Parameters

The ABET II software automatically collected the following parameters throughout the training and probe trial schedules in order to assess 5-CSRTT performance:

- Accuracy: number of correct responses divided by the sum of correct and incorrect responses ($\# \text{ correct} / (\# \text{ correct} + \# \text{ incorrect}) \times 100$). Accuracy is the main measure of sustained attention in this task.
- Omission: number of omissions divided by total number of trials.
- Premature Responses: total number of responses during the 5 seconds ITI divided by the total number of trials. Premature responses reflect impulsivity in this task.
- Perseverative Correct: total number of nose pokes in the reward tray after a correct response, but before collection of the reward, divided by the total number of trials. This is a measure of compulsivity/cognitive inflexibility in this task.
- Correct Response Latency: average time it takes for the mouse to touch the screen after the onset of the stimulus. The correct response latency reflects cognitive processing speed in this task.
- Reward Collection Latency: average time taken after a correct response to the collection of the milkshake reward from the reward tray.

Statistical Analysis: All stats were performed using Graphpad Prism 6.0 software. Comparisons between experimental groups were made by either a Student's t-test or two-way ANOVA with repeated measures. For that, the data passed normal distribution tested by the software. When appropriate a Tukey's *post hoc* comparison test was used. In all comparisons, $p < 0.05$ was considered statistically significant and all data are expressed as mean \pm SEM.

3 Results

3.1 Training of 3xTG and 5xFAD mice in the 5-CSRTT

The performance of both the 3xTG and 5xFAD males and females did not differ from that of control mice for touchscreen operation training (pre-training phases). The number of trials (days) required to reach criteria across all pre-training phases did not differ between genotypes. Similarly, when mice were trained to respond to flashes of light displayed in one of the five spatial locations on the touchscreens (training phase), performance of 3xTG males did not differ from that of controls in reaching criteria during the 5-CSRTT training schedules of 4 seconds and 2 seconds (**Figure 4A**; 4 seconds: $t_{(22)} = 0.7002$, $p = 0.4912$; 2 seconds: $t_{(18)} = 1.456$, $p = 0.1625$). Interestingly, 3xTG female mice showed a significantly worse performance compared to controls during the 2 seconds schedule, needing more sessions to reach criteria, but were not different during the 4 seconds schedule (**Figure 4B**; 4 seconds: $t_{(19)} = 0.3997$, $p = 0.6938$; 2 seconds: $t_{(19)} = 0.2.121$, $p = 0.0473$). In contrast, performance of both males and females 5xFAD mice did not differ from that of controls during the 4 seconds and 2 seconds training schedules (**Figure 4C**; 4 seconds: $t_{(22)} = 0.3347$, $p = 0.7410$; 2 seconds: $t_{(22)} = 0.0325$, $p = 0.9744$; **Figure 4D**; 4 seconds: $t_{(21)} = 0.6643$, $p = 0.5137$; 2 seconds: $t_{(21)} = 0.9467$, $p = 0.3546$).

3.2 Attention Capacity of 3xTG Male Mice

We investigated the performance in the 5-CSRTT for 3xTG mice longitudinally to fully capture age-dependent deficits that are triggered by AD-like pathology. Based on

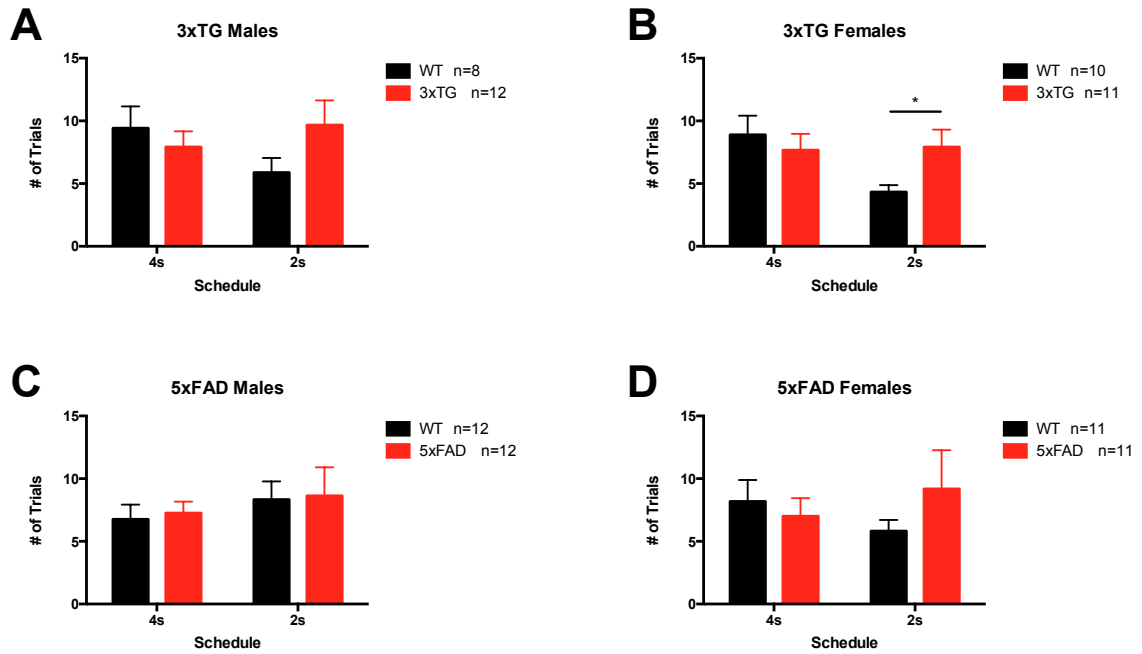


Figure 4: Trials to reach criteria in the 5-CSRTT for the 2 seconds and 4 seconds training schedules. A, 3xTG males and age-matched WT controls. B, 3xTG females and age-matched WT controls. C, 5xFAD males and age-matched WT controls. D, 5xFAD females and age-matched WT controls. (All data are \pm SEM, * p <0.05).

pathology accumulation and previous behavioural experiments (Romberg et al., 2011), we chose 4.5, 7 and 10 months to study 3xTG AD-mice.

Male 3xTG demonstrated deficits in accuracy, the main measure of attention in the 5-CSRTT, compared to age-matched control mice with an overall genotype difference in accuracy at 4.5 months (**Figure 5A**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 9.842$, $p = 0.0060$; main effect of stimulus duration, $F_{(3,51)} = 25.55$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 0.8163$, $p = 0.4908$). Compared to age-matched controls, 4.5 month old male 3xTG mice did not show a difference in omissions when compared to controls (**Figure 5B**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.0018$, $p = 0.9663$; main effect of stimulus duration, $F_{(3,51)} = 22.88$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 0.4374$, $p = 0.7272$). However, transgenic mice presented a difference in correct response latency, a measure of cognitive processing speed, when compared to controls (**Figure 5C**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 11.10$, $p = 0.0040$; main effect of stimulus duration, $F_{(3,51)} = 2.804$, $p = 0.049$; no interaction effect, $F_{(3,51)} = 0.4679$, $p = 0.7059$). There is no difference between genotypes for premature responses (**Figure 5D**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 2.228$, $p = 0.1538$; no effect of stimulus duration, $F_{(3,51)} = 1.354$, $p = 0.2673$; no interaction effect, $F_{(3,51)} = 0.3297$, $p = 0.78039$), number of perseverative correct responses (**Figure 5E**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.0828$, $p = 0.7770$; main effect of stimulus duration, $F_{(3,51)} = 3.506$, $p = 0.0218$; no interaction effect, $F_{(3,51)} = 1.883$, $p = 0.1441$), or reward collection latency (**Figure 5F**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 1.351$, $p = 0.2621$; no effect of stimulus duration, $F_{(3,51)} = 0.7123$, $p = 0.5492$; no interaction effect, $F_{(3,51)} = 1.165$, $p = 0.3321$).

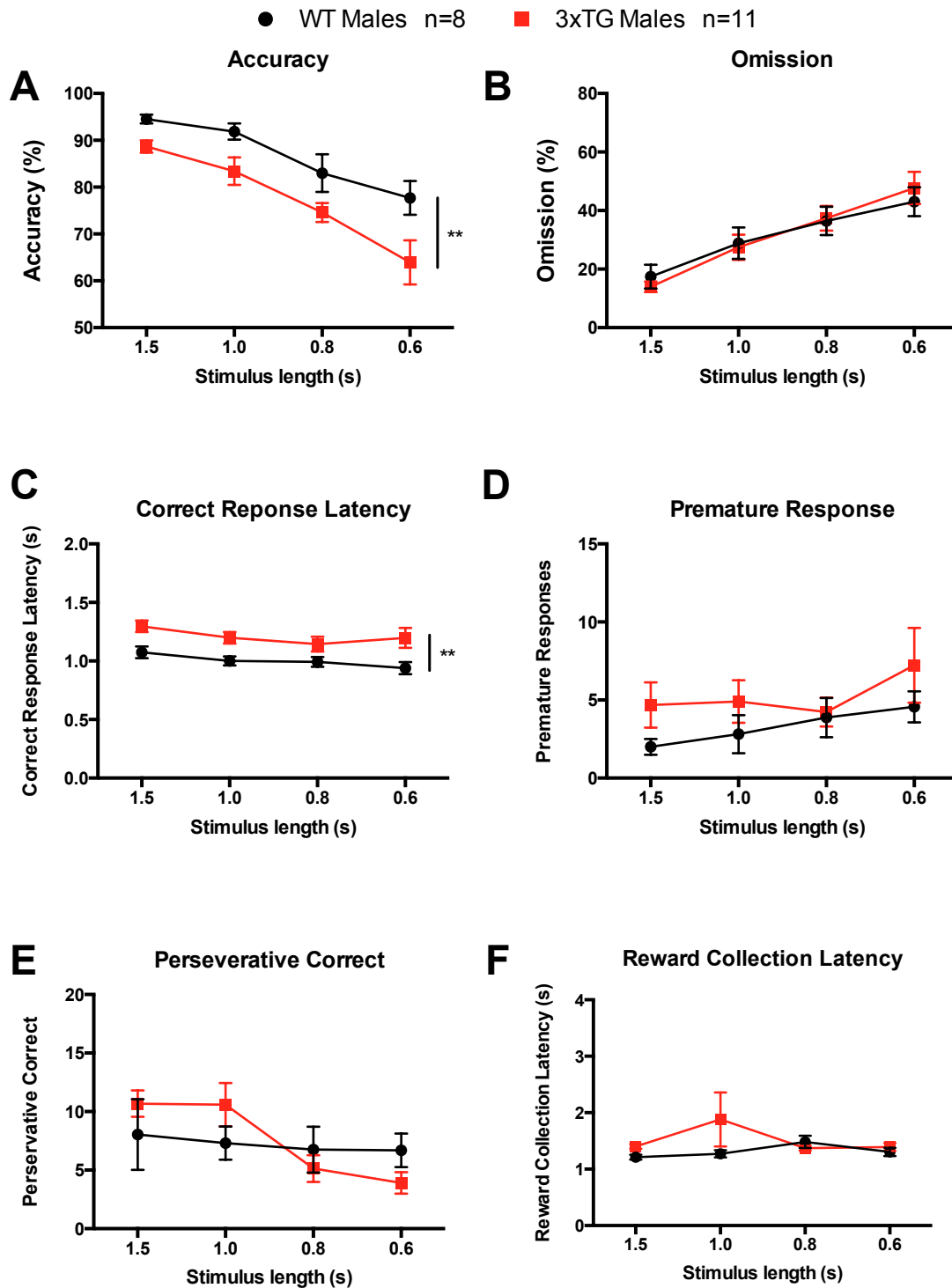


Figure 5: Performance and response measures of 4.5 month-old 3xTG Males tested at University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. A, Mean accuracy. B, Mean omission. C, Mean correct response latency. D, Mean premature responses. E, Mean perseverative correct. F, Mean reward collection latency (3xTG males n=11, WT males n=8, data are mean \pm SEM, **p<0.01)

At 7 months, male 3xTG mice showed a difference in accuracy compared to age-matched control littermates (**Figure 6A**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 4.385$, $p = 0.0516$; main effect of stimulus duration, $F_{(3,51)} = 26.74$, $p < 0.0001$; main interaction effect, $F_{(3,51)} = 3.092$, $p = 0.0351$) and *post-hoc* analysis revealed that they had a significantly lower accuracy at the 0.6s stimulus duration during the probe trials (**Fig. 6A**). Similarly to the 4.5 month-old transgenic mice, there were no differences between 3xTG and controls in omissions (**Figure 6B**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.5091$, $p = 0.4852$; main effect of stimulus duration, $F_{(3,51)} = 26.35$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 0.1061$, $p = 0.9562$). The 7 month old transgenic males also had significantly higher correct response latencies when compared to age-matched controls (**Figure 6C**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 9.434$, $p = 0.0069$; main effect of stimulus duration, $F_{(3,51)} = 4.252$, $p = 0.0093$; no interaction effect, $F_{(3,51)} = 0.5739$, $p = 0.6348$). There was no difference between genotypes in premature responses (**Figure 6D**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 2.076$, $p = 0.1677$; main effect of stimulus duration, $F_{(3,51)} = 3.532$, $p = 0.0211$; no interaction effect, $F_{(3,51)} = 0.2442$, $p = 0.8651$), perseverative correct responses (**Figure 6E**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.4685$, $p = 0.5029$; main effect of stimulus duration, $F_{(3,51)} = 4.866$, $p = 0.0047$; no interaction effect, $F_{(3,51)} = 0.7868$, $p = 0.5068$), or reward collection latency (**Figure 6F**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.9133$, $p = 0.3526$; no effect of stimulus duration, $F_{(3,51)} = 1.391$, $p = 0.2561$; main interaction effect, $F_{(3,51)} = 2.117$, $p = 0.1095$).

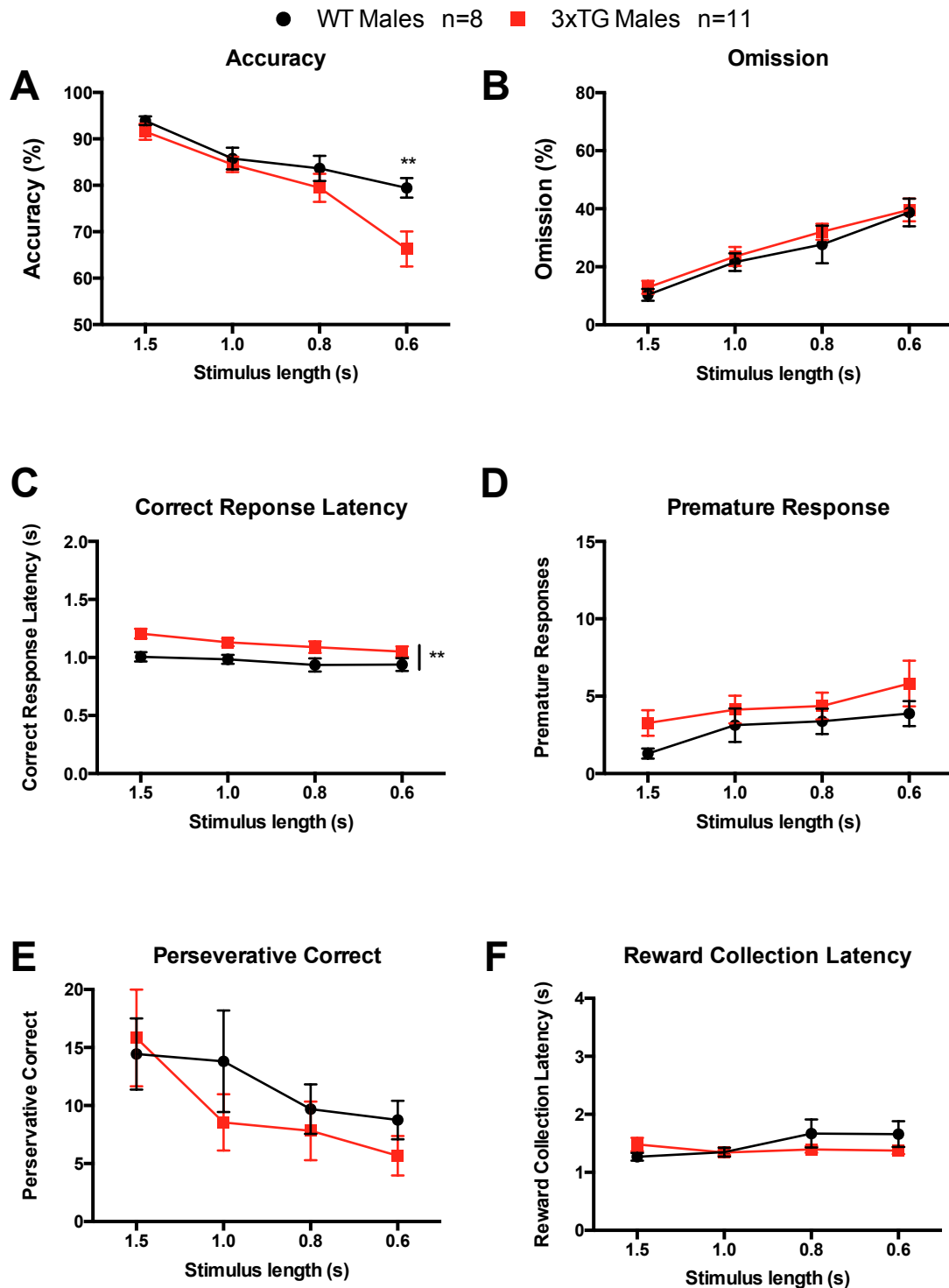


Figure 6. Performance and response measures of 7 month-old 3xTG Males at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG males n=11, WT males n=8, data are mean \pm SEM, **p<0.01)

Ten month old 3xTG males showed both a genotype and interaction difference in accuracy compared to age-matched controls (**Figure 7A**; RM-ANOVA, main effect of genotype, $F_{(1,13)} = 5.261$, $p = 0.0391$; main effect of stimulus duration, $F_{(3,39)} = 19.81$, $p < 0.0001$; main interaction effect, $F_{(3,39)} = 4.661$, $p = 0.0071$). *Post-hoc* analysis revealed that they had significantly lower accuracy at the 0.6s stimulus duration during the probe trials (**Fig. 7A**). Moreover, the 10 months old 3xTG males also presented a difference in omissions during the task when compared to controls (**Figure 7B**; RM-ANOVA, main effect of genotype, $F_{(1,13)} = 9.371$, $p = 0.0091$; main effect of stimulus duration, $F_{(3,39)} = 16.91$, $p < 0.0001$; no interaction effect, $F_{(3,39)} = 2.4111$, $p = 0.0815$). Male 3xTG mice also had a significant difference in correct response latency at this age (**Figure 7C**; RM-ANOVA, main effect of genotype, $F_{(1,13)} = 4.709$, $p = 0.0491$; no effect of stimulus duration, $F_{(3,39)} = 0.5234$, $p = 0.6687$; no interaction effect, $F_{(3,39)} = 0.3645$, $p = 0.7790$). In contrast, the 10 month old 3xTG mice did not reveal a difference in premature responses between the two genotypes (**Figure 7D**; RM-ANOVA, no effect of genotype, $F_{(1,13)} = 0.1053$, $p = 0.7507$; no effect of stimulus duration, $F_{(3,39)} = 1.057$, $p = 0.3782$; no interaction effect, $F_{(3,39)} = 1.760$, $p = 0.1708$). Perseverative correct, which reflects compulsivity, was different between genotypes (**Figure 7E**; RM-ANOVA, main effect of genotype, $F_{(1,13)} = 5.649$, $p = 0.0335$; no effect of stimulus duration, $F_{(3,39)} = 1.676$, $p = 0.1880$; no interaction effect, $F_{(3,39)} = 0.4492$, $p = 0.7193$). Lastly, reward collection latency was not different between genotypes (**Figure 7F**; RM-ANOVA, no effect of genotype, $F_{(1,13)} = 0.9227$, $p = 0.3543$; no effect of stimulus duration, $F_{(3,39)} = 1.678$, $p = 0.1876$; no interaction effect, $F_{(3,39)} = 1.621$, $p = 0.2000$).

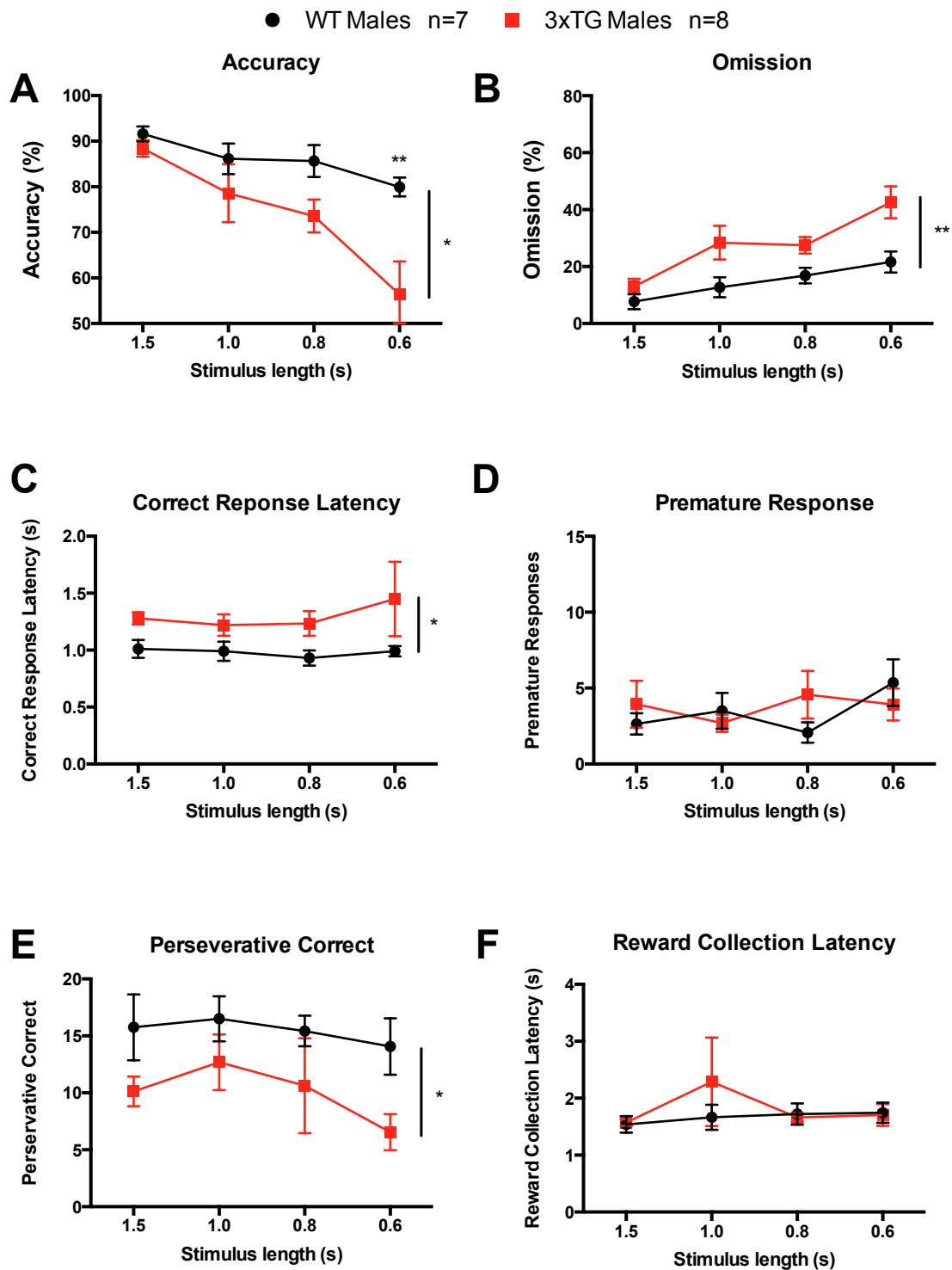


Figure 7. Performance and response measures of 10 month-old 3xTG Males at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. Mice were subject to a series of probe trials and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG males n=8, WT males n=7, data are mean \pm SEM, * p <0.05 and ** p <0.01)

3.3 Attention Capacity in 3xTG Female Mice

We also investigated the performance of female 3xTG mice longitudinally in the 5-CSRTT. Similar to males, female 3xTG mice also revealed differences in both accuracy and correct response latencies throughout all three probe trials.

The 4.5 month old 3xTG females showed decreased accuracy compared to controls (**Figure 8A**; RM-ANOVA, main effect of genotype, $F_{(1,18)} = 4.523$, $p = 0.0475$; main effect of stimulus duration, $F_{(3,54)} = 18.79$, $p < 0.0001$; no interaction effect, $F_{(3,54)} = 1.593$, $p = 0.2017$) and increased omissions (**Figure 8B**; RM-ANOVA, no effect of genotype, $F_{(1,18)} = 3.604$, $p = 0.0738$; main effect of stimulus duration, $F_{(3,54)} = 69.94$, $p < 0.0001$; main interaction effect, $F_{(3,54)} = 2.936$, $p = 0.0414$). *Post-hoc* analysis revealed a significant increase in omissions at the 0.6s stimulus duration (**Fig. 8B**). In addition, the transgenic females showed both a genotype and interaction difference in correct response latency (**Figure 8C**; RM-ANOVA, main effect of genotype, $F_{(1,18)} = 12.44$, $p = 0.0024$; no effect of stimulus duration, $F_{(3,54)} = 1.490$, $p = 0.2276$; main interaction effect, $F_{(3,54)} = 3.772$, $p = 0.0157$) with *post-hoc* analysis revealing a significant increase in latency at the 0.6s stimulus duration (**Fig. 8C**). There were no other differences between 4.5 months old 3xTG females and age-matched controls during the first probe trial in premature responses (**Figure 8D**; RM-ANOVA, no effect of genotype, $F_{(1,18)} = 3.886$, $p = 0.0643$;

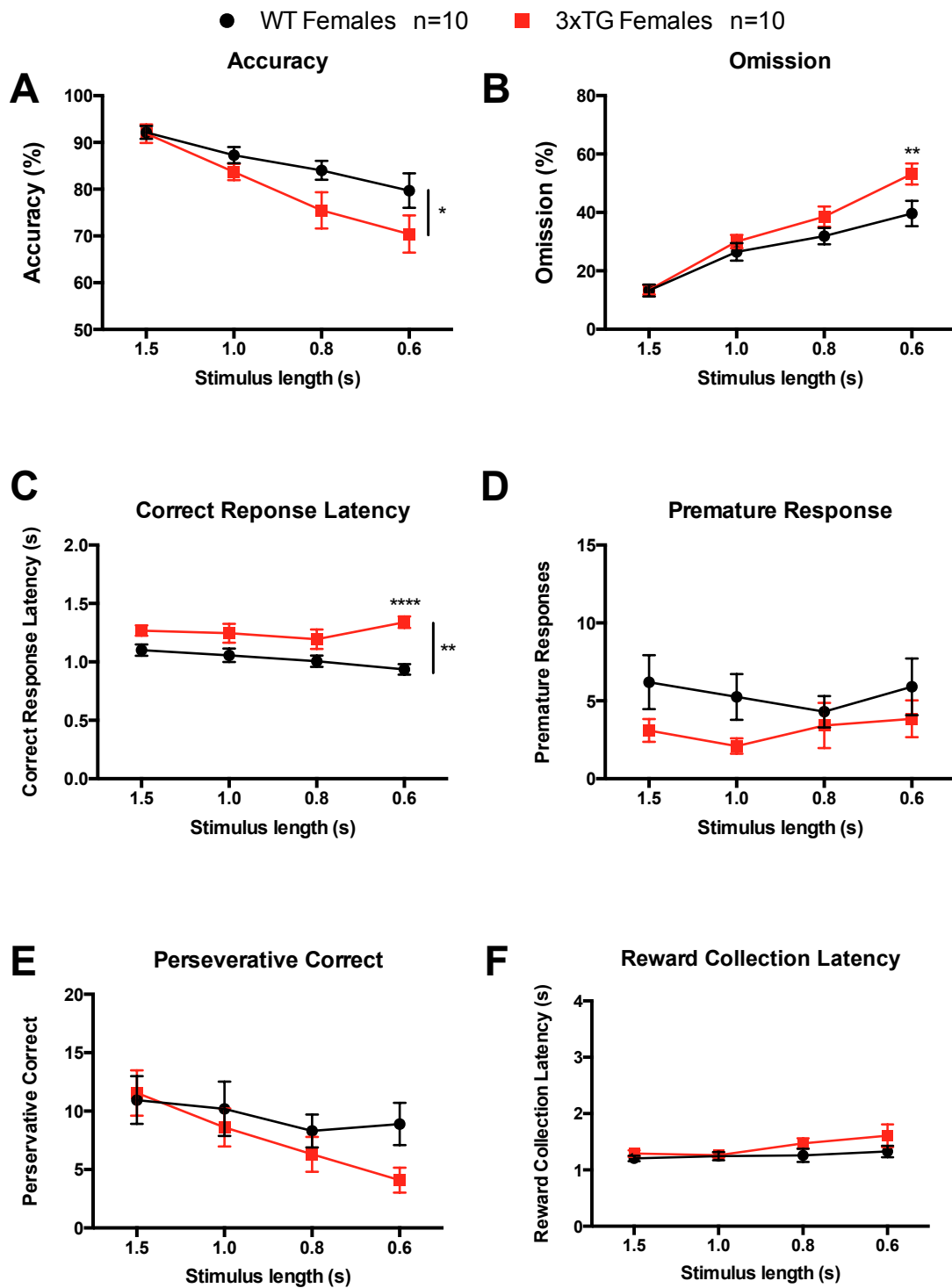


Figure 8. Performance and response measures of 4.5 month-old 3xTG Females at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG females n=10, WT females n=10, data are mean \pm SEM, * p <0.05, ** p <0.01 and **** p <0.0001)

no effect of stimulus duration, $F_{(3,54)} = 0.4987, p = 0.6848$; no interaction effect, $F_{(3,54)} = 0.4138, p = 0.7437$) perseverative correct (**Figure 8E**; RM-ANOVA, no effect of genotype, $F_{(1,18)} = 1.127, p = 0.3025$; main effect of stimulus duration, $F_{(3,54)} = 4.963, p = 0.0041$; no interaction effect, $F_{(3,54)} = 1.330, p = 0.2742$), or reward collection latency (**Figure 8F**; RM-ANOVA, no effect of genotype, $F_{(1,18)} = 2.551, p = 0.1276$; no effect of stimulus duration, $F_{(3,54)} = 2.590, p = 0.0622$; no interaction effect, $F_{(3,54)} = 0.8387, p = 0.4786$).

Seven month-old 3xTG females showed a significantly lower accuracy compared to age-matched controls (**Figure 9A**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 10.48, p = 0.0048$; main effect of stimulus duration, $F_{(3,51)} = 27.11, p < 0.0001$; no interaction effect, $F_{(3,51)} = 1.860, p = 0.1481$). In addition, they also had higher omissions (**Figure 9B**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 4.890, p = 0.0410$; main effect of stimulus duration, $F_{(3,51)} = 19.63, p < 0.0001$; no interaction effect, $F_{(3,51)} = 0.9319, p = 0.14321$) and correct response latencies (**Figure 9C**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 10.35, p = 0.0051$; no effect of stimulus duration, $F_{(3,51)} = 0.4251, p = 0.7358$; no interaction effect, $F_{(3,51)} = 0.3717, p = 0.7737$). There were no differences between the genotypes in premature responses (**Figure 9D**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.3622, p = 0.5552$; main effect of stimulus duration, $F_{(3,51)} = 2.890, p = 0.0433$; no interaction effect, $F_{(3,51)} = 0.7168, p = 0.5466$), perseverative correct responses (**Figure 9E**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 6.392, p = 0.0517$; main effect of stimulus duration, $F_{(3,51)} = 4.773, p = 0.0052$; no interaction effect, $F_{(3,51)} = 0.0653, p = 0.978$) or reward collection latencies (**Figure 9F**; RM-ANOVA, no effect of genotype,

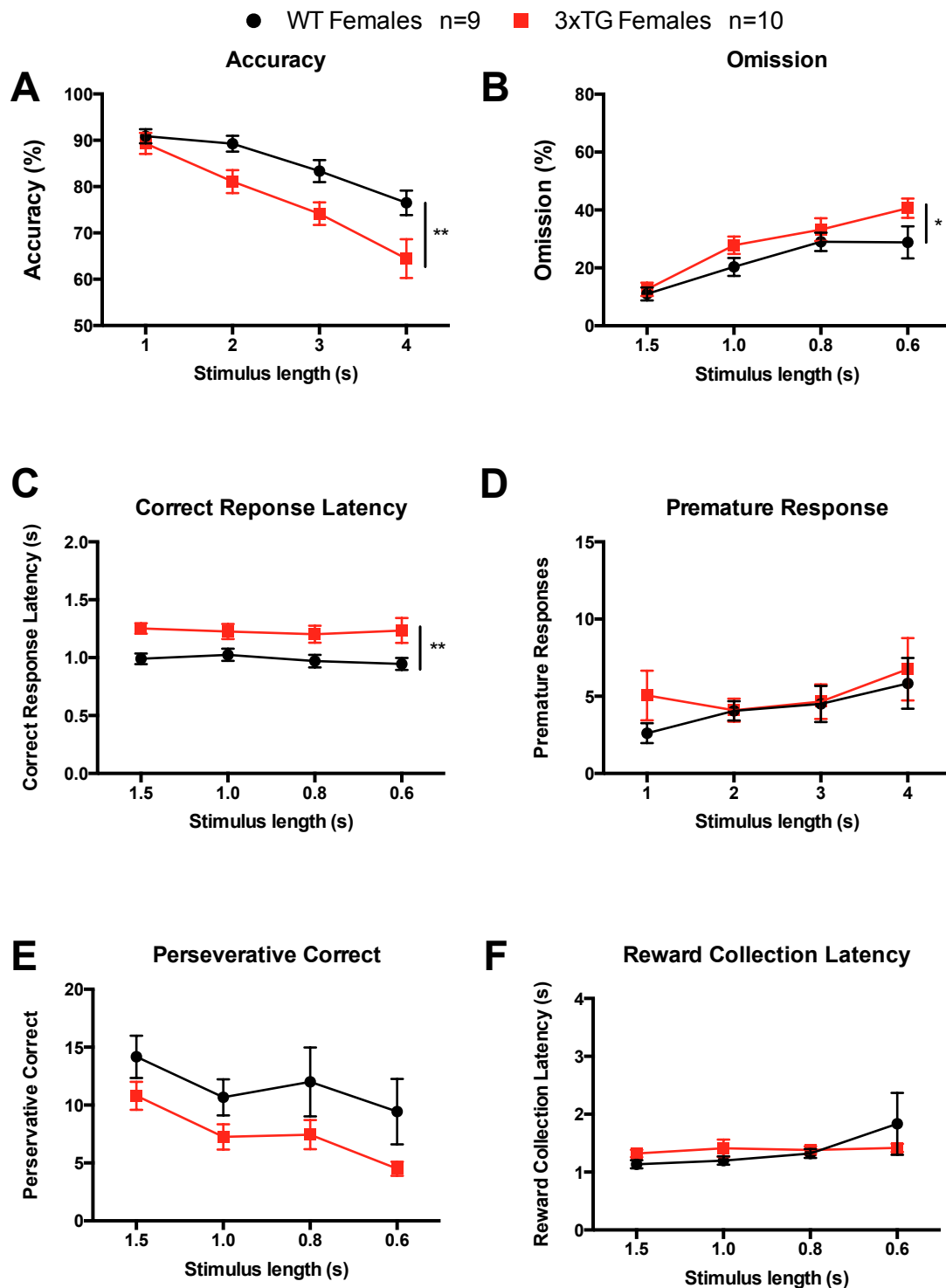


Figure 9. Performance and response measures of 7 month-old 3xTG Females at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG females n=10, WT females n=9, data are mean \pm SEM, * p <0.05 and ** p <0.01)

$F_{(1,17)} = 0.0068, p = 0.9353$; no effect of stimulus duration, $F_{(3,51)} = 1.579, p = 0.2058$; no interaction effect, $F_{(3,51)} = 1.118, p = 0.3506$).

Surprisingly, 10 month-old 3xTG females did not show a significant difference in accuracy when compared to controls (**Figure 10A**; RM-ANOVA, no effect of genotype, $F_{(1,16)} = 2.992, p = 0.1029$; main effect of stimulus duration, $F_{(3,48)} = 38.54, p < 0.0001$; no interaction effect, $F_{(3,48)} = 0.7550, p = 0.5249$), or omissions (**Figure 10B**; RM-ANOVA, no effect of genotype, $F_{(1,16)} = 3.080, p = 0.0984$; main effect of stimulus duration, $F_{(3,48)} = 29.15, p < 0.0001$; no interaction effect, $F_{(3,48)} = 1.189, p = 0.3237$). The 3xTG females did show a higher correct response latency compared to controls (**Figure 10C**; RM-ANOVA, main effect of genotype, $F_{(1,16)} = 13.95, p = 0.0018$; no effect of stimulus duration, $F_{(3,48)} = 0.8806, p = 0.4578$; no interaction effect, $F_{(3,48)} = 1.028, p = 0.3885$). However, no differences were observed between the 10 month-old genotypes in premature responses (**Figure 10D**; RM-ANOVA, no effect of genotype, $F_{(1,16)} = 0.8635, p = 0.3666$; main effect of stimulus duration, $F_{(3,48)} = 3.301, p = 0.0280$; no interaction effect, $F_{(3,48)} = 0.5091, p = 0.6779$), perseverative correct (**Figure 10E**; RM-ANOVA, no effect of genotype, $F_{(1,16)} = 3.542, p = 0.0817$; main effect of stimulus duration, $F_{(3,48)} = 4.275, p = 0.0094$; no interaction effect, $F_{(3,48)} = 1293, p = 0.2876$), or reward collection latency (**Figure 10F**; RM-ANOVA, no effect of genotype, $F_{(1,16)} = 0.0590, p = 0.8111$; main effect of stimulus duration, $F_{(3,48)} = 1.208, p = 0.3170$; no interaction effect, $F_{(3,48)} = 1.118, p = 0.3510$).

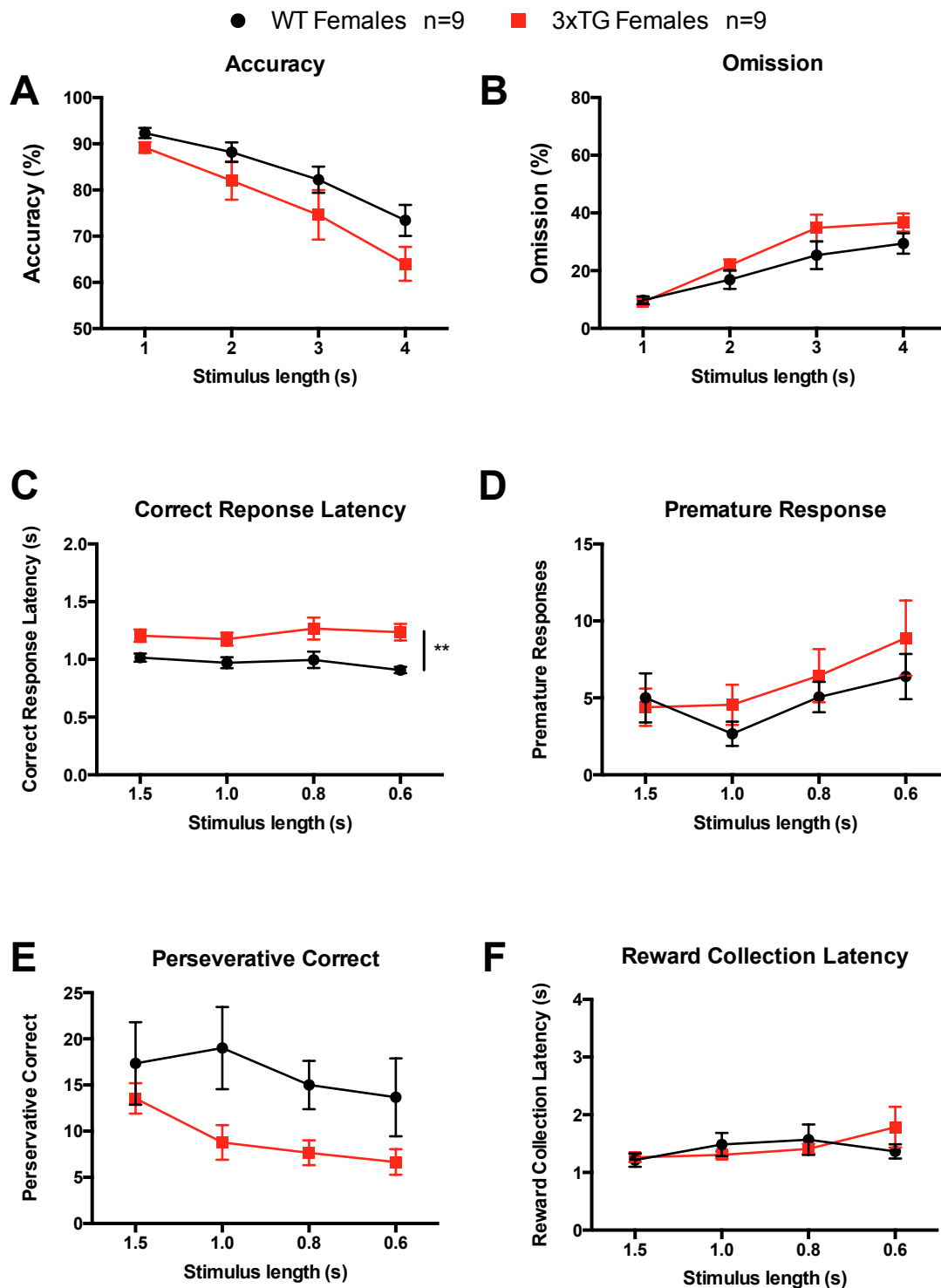


Figure 10. Performance and response measures of 10 month-old 3xTG Females at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG females n=9, WT females n=9, data are mean \pm SEM, **p<0.01)

3.4 Reproducibility of 3xTG Data Between Different Sites

In order to test for reproducibility of data using the 5-CSRTT, age-matched 3xTG mice and controls were run at two separate sites, at the University of Western Ontario (site 1) and at the University of Guelph (site 2). Due to the potential for the greatest age-dependent deficits related to AD-pathology, the accuracy data for the 10 month-old mice was analyzed to determine whether data obtained with touchscreens are indeed reproducible. For that we obtained data from Dr. Boyer Winters' laboratory (Data obtained by the PhD student Daniel Palmer) who collaborates in this project. It should be noted that the results we obtained with 3xTG male mice in site 1 were already reproducing data previously published for these mice in touchscreens (Romberg et al 2011). No difference was observed in performance of 10 month-old wild-type males between sites (**Figure 11A**; RM-ANOVA, no effect of genotype, $F_{(1,15)} = 1.153$, $p = 0.2999$; main effect of stimulus duration, $F_{(3,45)} = 13.86$, $p < 0.0001$; no interaction effect, $F_{(3,45)} = 0.3881$, $p = 0.7621$) and no difference was observed in performance of 10 month-old 3xTG males between sites (**Figure 11B**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 2.362$, $p = 0.1466$; main effect of stimulus duration, $F_{(3,42)} = 28.68$, $p < 0.0001$; no interaction effect, $F_{(3,42)} = 1.681$, $p = 0.1856$). Similarly, we observed no difference between sites in performance of female wild-type mice (**Figure 11C**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 0.9362$, $p = 0.3497$; main effect of stimulus duration, $F_{(3,42)} = 21.76$, $p < 0.0001$; no interaction effect, $F_{(3,42)} = 0.2185$, $p = 0.8830$), or the female 3xTG mice (**Figure 11D**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 1.614$, $p = 0.2210$; main effect of stimulus duration, $F_{(3,51)} = 41.20$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 0.3616$, $p = 0.7810$). Despite some minor differences when we compared other

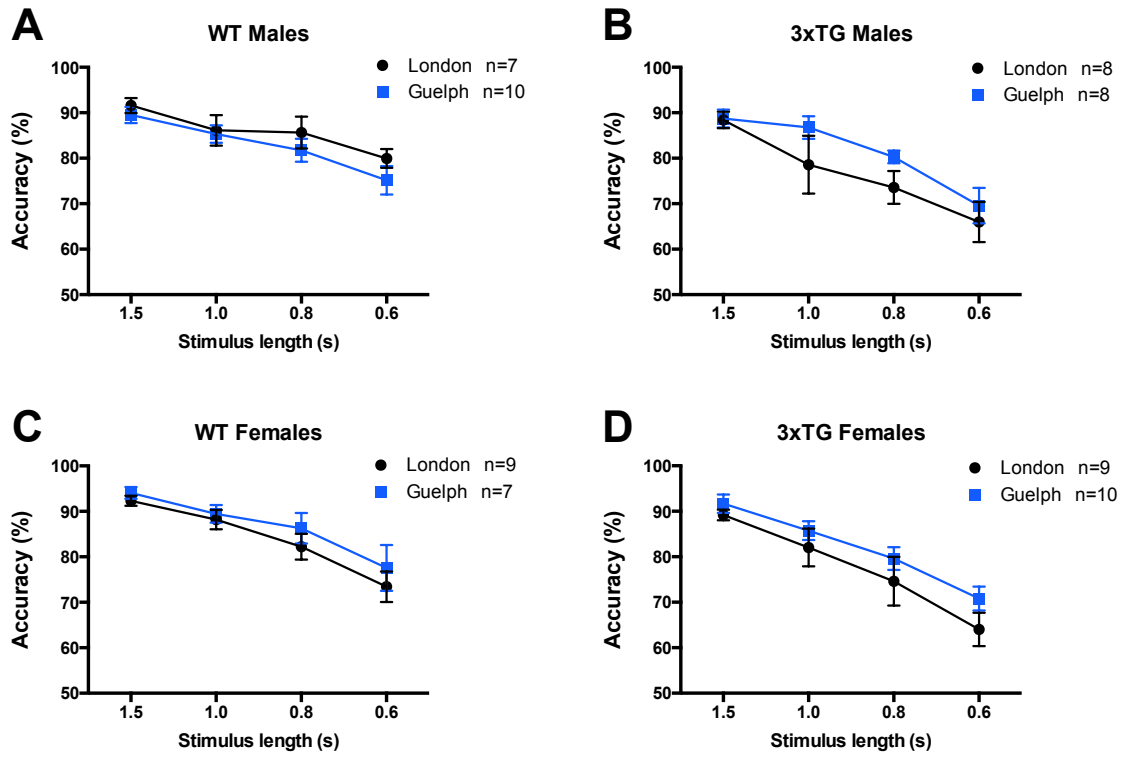


Figure 11. Comparison of the performance and response measures of 10 month-old 3xTG and Wild-type Males and Females tested at the University of Western Ontario (London) and at the University of Guelph (Guelph) during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy for WT males. **B**, Mean accuracy for 3xTG males from both sites. **C**, Mean accuracy for WT females. **D**, Mean accuracy for 3xTG females. (Data are mean \pm SEM).

parameters in all the ages, the results were mostly reproducible between sites (data not shown). Since there were no differences within groups of control and 3xTG mice, we analysed data between the two sites for all ages in order to increase statistical power and define robust age-dependent AD-related phenotypes in the 5-CSRTT touchscreen tests for this mouse line.

Our analysis reveals that 3xTG male mice presented attention deficits when data were combined with decreased accuracy of 4.5 month-old mice (**Figure 12A**; RM-ANOVA, main effect of genotype, $F_{(1,38)} = 5.176$, $p = 0.0286$; main effect of stimulus duration, $F_{(3,114)} = 59.45$, $p < 0.0001$; no interaction effect, $F_{(3,114)} = 0.8496$, $p = 0.4696$), however we did not observe a difference in omissions (**Figure 12B**; RM-ANOVA, no effect of genotype, $F_{(1,38)} = 0.2818$, $p = 0.5986$; main effect of stimulus duration, $F_{(3,114)} = 61.76$, $p < 0.0001$; no interaction effect, $F_{(3,114)} = 0.3983$, $p = 0.7545$). The combined data also demonstrated a difference in correct response latency between genotypes (**Figure 12C**; RM-ANOVA, main effect of genotype, $F_{(1,38)} = 13.44$, $p = 0.0008$; main effect of stimulus duration, $F_{(3,114)} = 10.70$, $p < 0.0001$; no interaction effect, $F_{(3,114)} = 0.4505$, $p = 0.7174$). No differences were observed between the combined 4.5 month-old 3xTG males and controls in premature responses (**Figure 12D**; RM-ANOVA, no effect of genotype, $F_{(1,38)} = 0.1597$, $p = 0.6917$; main effect of stimulus duration, $F_{(3,114)} = 3.496$, $p = 0.0186$; no interaction effect, $F_{(3,114)} = 0.0727$, $p = 0.9745$), perseverative correct (**Figure 12E**; RM-ANOVA, no effect of genotype, $F_{(1,38)} = 1.989$, $p = 0.1666$; main effect of stimulus duration, $F_{(3,114)} = 9.698$, $p < 0.0001$; no interaction effect, $F_{(3,114)} = 1.845$, $p = 0.1430$), or reward collection latency (**Figure 12F**; RM-ANOVA, no effect of genotype, $F_{(1,38)} =$

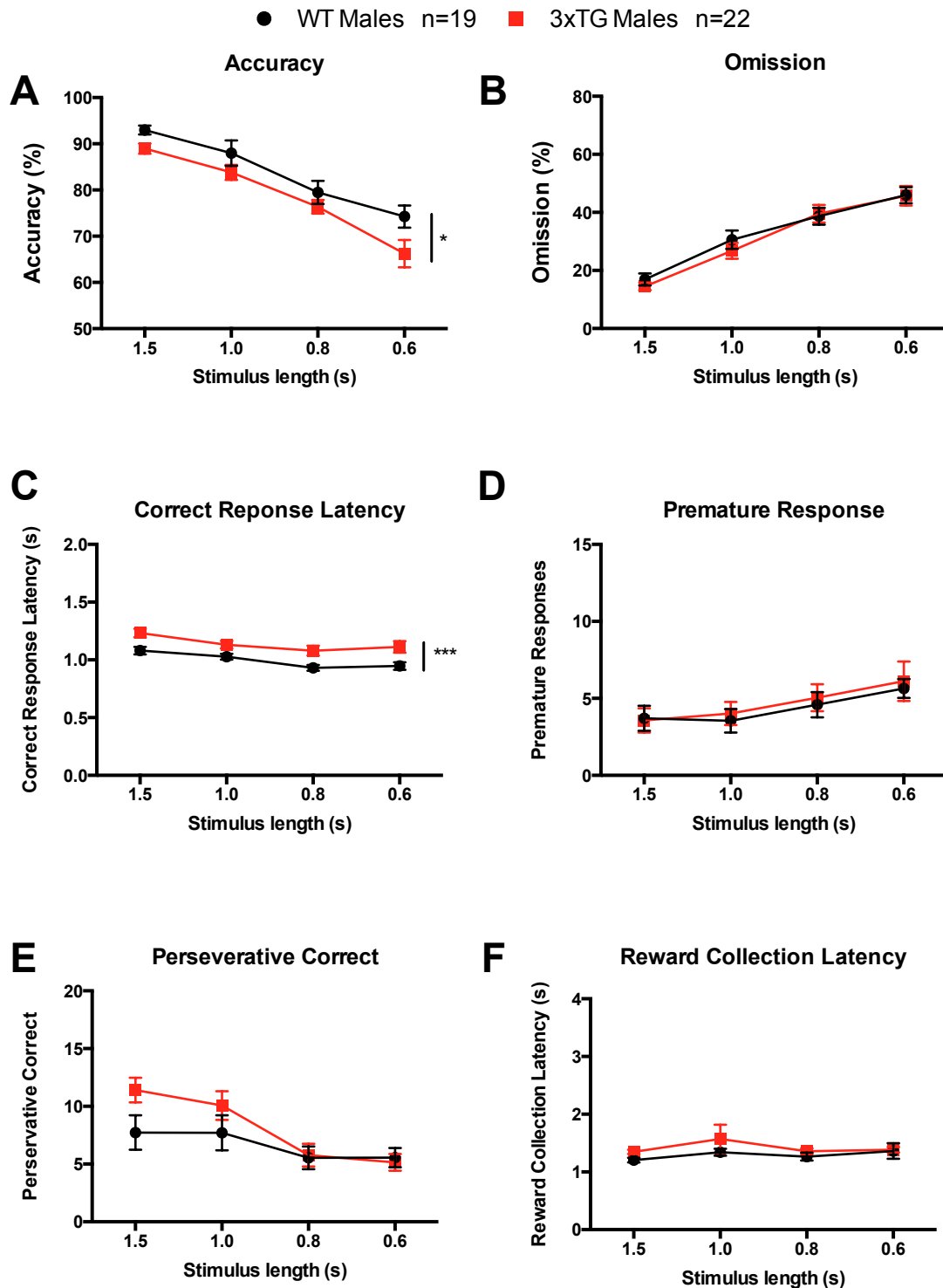


Figure 12. Performance and response measures of the combined data for 4.5 month-old 3xTG Males at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG males n=22, WT males n=19, data are mean \pm SEM, * p <0.05 and *** p <0.001)

1.899, $p = 0.1762$; no effect of stimulus duration, $F_{(3,114)} = 1.049$, $p = 0.3740$; no interaction effect, $F_{(3,114)} = 0.3240$, $p = 0.8080$).

Combined data obtained with 7 month-old 3xTG and control male mice showed both a genotype and interaction difference in accuracy (**Figure 13A**; RM-ANOVA, main effect of genotype, $F_{(1,31)} = 7.876$, $p = 0.0086$; main effect of stimulus duration, $F_{(3,93)} = 48.29$, $p < 0.0001$; main interaction effect, $F_{(3,93)} = 3.546$, $p = 0.0175$) and a *Post-hoc* analysis revealed a significant difference between at the 0.6s stimulus duration (**Fig. 13A**). There were no differences observed for omissions (**Figure 13B**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 0.9227$, $p = 0.3442$; main effect of stimulus duration, $F_{(3,93)} = 59.83$, $p < 0.0001$; no interaction effect, $F_{(3,93)} = 0.0046$, $p = 0.9996$). The combined data demonstrated that 7 month-old 3xTG males continued to have higher correct response latencies compared to age-matched controls (**Figure 13C**; RM-ANOVA, main effect of genotype, $F_{(1,31)} = 14.80$, $p = 0.0006$; main effect of stimulus duration, $F_{(3,93)} = 10.44$, $p < 0.0001$; no interaction effect, $F_{(3,93)} = 1.077$, $p = 0.3692$). No difference in performance was observed in premature responses (**Figure 13D**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 4802$, $p = 0.4935$; main effect of stimulus duration, $F_{(3,93)} = 3.449$, $p = 0.0198$; no interaction effect, $F_{(3,93)} = 0.6072$, $p = 0.6120$), number of perseverative correct responses (**Figure 13E**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 0.0082$, $p = 0.9285$; main effect of stimulus duration, $F_{(3,93)} = 5.594$, $p = 0.0014$; no interaction effect, $F_{(3,93)} = 0.8452$, $p = 0.4726$), or reward collection latency (**Figure 13F**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 0.2646$, $p = 0.6107$; no effect of stimulus duration, $F_{(3,93)} = 1.553$, $p = 0.2060$; no interaction effect, $F_{(3,93)} = 1.904$, $p = 0.0889$).

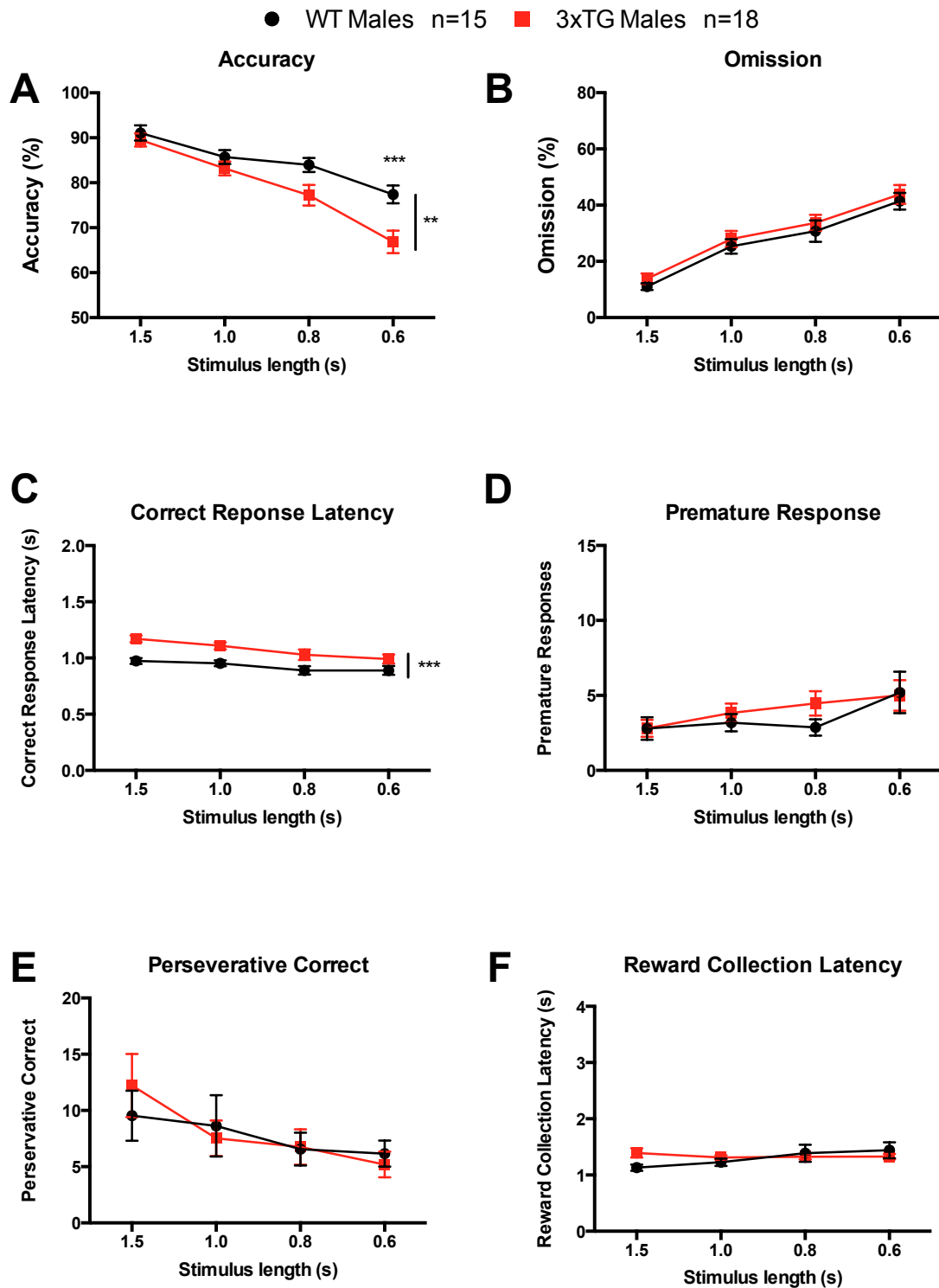


Figure 13. Performance and response measures of the combined data for 7 month-old 3xTG Males at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG males n=18, WT males n=15, data are mean \pm SEM, **p<0.01 and ***p<0.001)

At 10 months of age, the combined data demonstrated that male 3xTG mice continued to display both a genotype and interaction difference in accuracy (**Figure 14A**; RM-ANOVA, main effect of genotype, $F_{(1,31)} = 5.459$, $p = 0.0261$; main effect of stimulus duration, $F_{(3,93)} = 43.61$, $p < 0.0001$; main interaction effect, $F_{(3,93)} = 5.000$, $p = 0.0029$). A *post-hoc* analysis revealed a difference between genotypes at the 0.6s stimulus duration (**Fig. 14A**). The data from both sites also revealed a difference in omissions when we compared the male mice from the two genotypes (**Figure 14B**; RM-ANOVA, main effect of genotype, $F_{(1,31)} = 4.610$, $p = 0.0397$; main effect of stimulus duration, $F_{(3,93)} = 43.38$, $p < 0.0001$; no interaction effect, $F_{(3,93)} = 1.794$, $p = 0.1538$). Likewise, the transgenic males continued to demonstrate increased correct response latency compared to controls (**Figure 14C**; RM-ANOVA, main effect of genotype, $F_{(1,31)} = 7.398$, $p = 0.0106$; no effect of stimulus duration, $F_{(3,93)} = 0.4928$, $p = 0.6882$; no interaction effect, $F_{(3,93)} = 0.6931$, $p = 0.5585$). However, no differences in premature responses (**Figure 14D**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 0.0664$, $p = 0.7983$; no effect of stimulus duration, $F_{(3,93)} = 1.613$, $p = 0.1917$; no interaction effect, $F_{(3,93)} = 0.2399$, $p = 0.8683$), perseverative correct (**Figure 14E**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 0.3807$, $p = 0.5417$; main effect of stimulus duration, $F_{(3,93)} = 3.643$, $p = 0.0194$; no interaction effect, $F_{(3,93)} = .4961$, $p = 0.6859$) or reward collection latency (**Figure 14F**; RM-ANOVA, no effect of genotype, $F_{(1,31)} = 0.2939$, $p = 0.5916$; no effect of stimulus duration, $F_{(3,93)} = 2.573$, $p = 0.0587$; no interaction effect, $F_{(3,93)} = 0.4875$, $p = 0.6918$) were observed in the data from both sites.

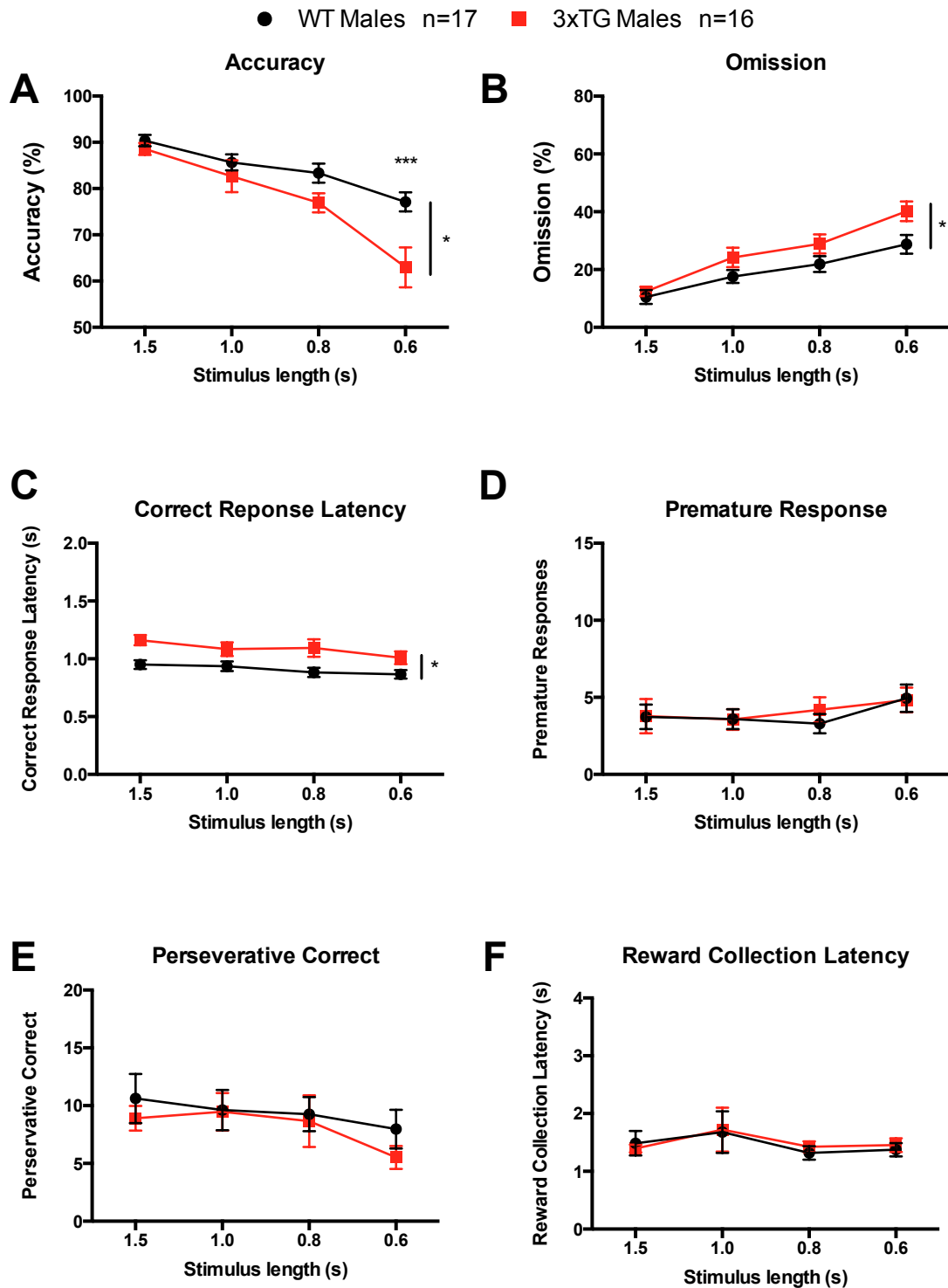


Figure 14. Performance and response measures of the combined data for 10 month-old 3xTG Males at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG males n=16, WT males n=17, data are mean \pm SEM, * p <0.05 and *** p <0.001)

The combination of data between sites also revealed the 3xTG females to have a significantly lower accuracy during the 4.5 month-old probes (**Figure 15A**; RM-ANOVA, main effect of genotype, $F_{(1,35)} = 6.358$, $p = 0.0164$; main effect of stimulus duration, $F_{(3,105)} = 48.35$, $p < 0.0001$; no interaction effect, $F_{(3,105)} = 0.8023$, $p = 0.4953$), but no difference in omissions (**Figure 15B**; RM-ANOVA, no effect of genotype, $F_{(1,35)} = 1.623$, $p = 0.2111$; main effect of stimulus duration, $F_{(3,105)} = 94.38$, $p < 0.0001$; no interaction effect, $F_{(3,105)} = 0.9048$, $p = 0.4415$). In addition, female 3xTG mice showed an increase in correct response latency (**Figure 15C**; RM-ANOVA, main effect of genotype, $F_{(1,35)} = 16.26$, $p = 0.0003$; main effect of stimulus duration, $F_{(3,105)} = 3.911$, $p = 0.0108$; no interaction effect, $F_{(3,105)} = 1.111$, $p = 0.3480$), and decreased premature responses (**Figure 15D**; RM-ANOVA, main effect of genotype, $F_{(1,35)} = 5.465$, $p = 0.0252$; no effect of stimulus duration, $F_{(3,105)} = 0.8707$, $p = 0.4588$; no interaction effect, $F_{(3,105)} = 0.1364$, $p = 0.9381$). The combined data for the 4.5 month-old 3xTG females and controls did not reveal a difference in perseverative correct responses (**Figure 15E**; RM-ANOVA, no effect of genotype, $F_{(1,35)} = 1.563$, $p = 0.2195$; main effect of stimulus duration, $F_{(3,105)} = 8.066$, $p < 0.0001$; no interaction effect, $F_{(3,105)} = 0.6851$, $p = 0.5631$), or reward collection latency (**Figure 15F**; RM-ANOVA, no effect of genotype, $F_{(1,35)} = 0.1229$, $p = 0.7280$; no effect of stimulus duration, $F_{(3,105)} = 1.947$, $p = 0.1266$; no interaction effect, $F_{(3,105)} = 0.8414$, $p = 0.4742$).

Combined data between sites for probe trials at 7 months of age showed 3xTG females to have deficits in accuracy compared to controls (**Figure 16A**; RM-ANOVA, main effect of genotype, $F_{(1,32)} = 6.825$, $p = 0.0136$; main effect of stimulus duration, $F_{(3,96)} = 52.40$, $p < 0.0001$; no interaction effect, $F_{(3,96)} = 0.8580$, $p = 0.4657$). Interestingly, the combined

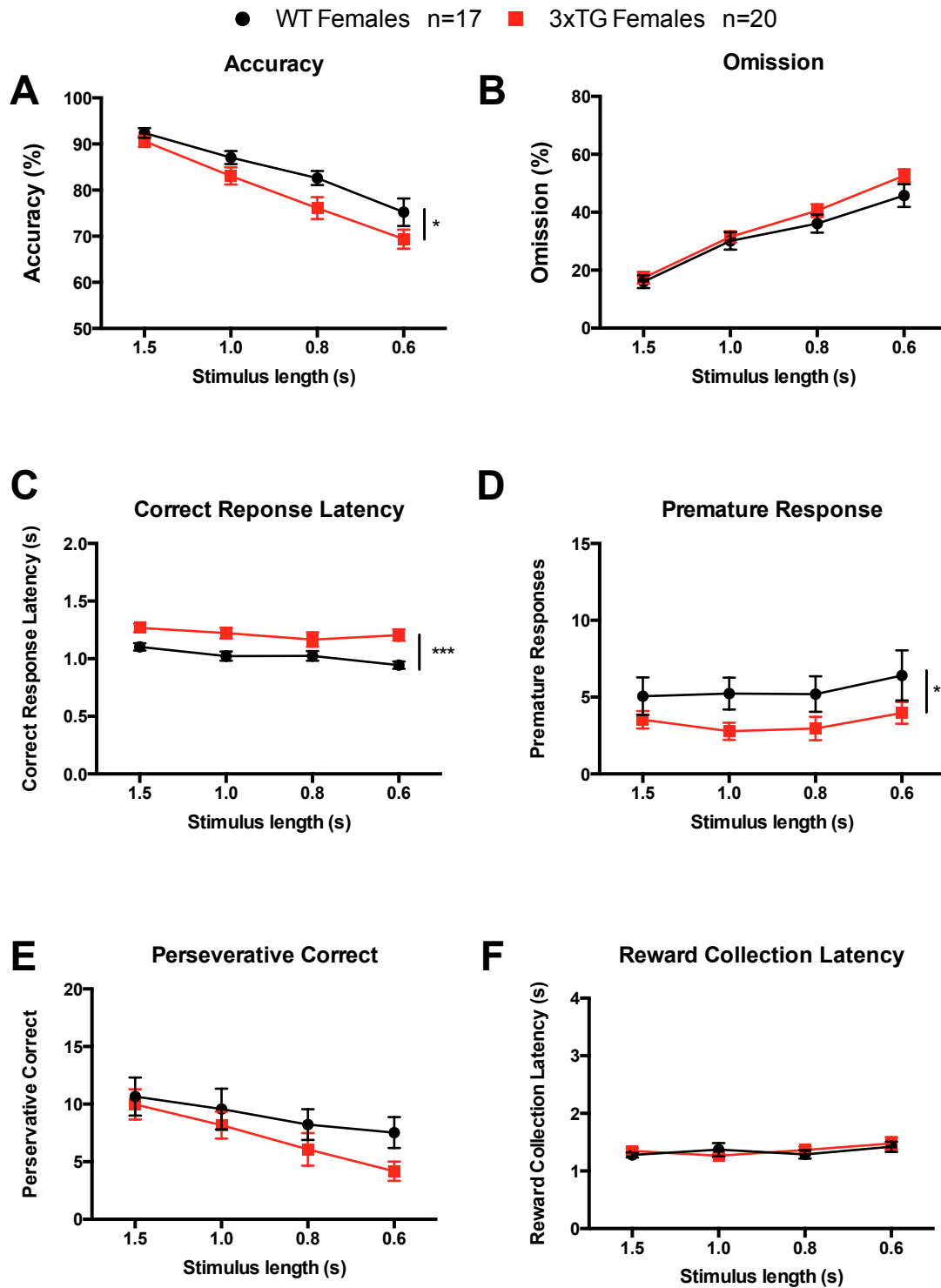


Figure 15. Performance and response measures of the combined data for 4.5 month-old 3xTG Females at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG females n=20, WT females n=17, data are mean \pm SEM, * p <0.05 and *** p <0.001)

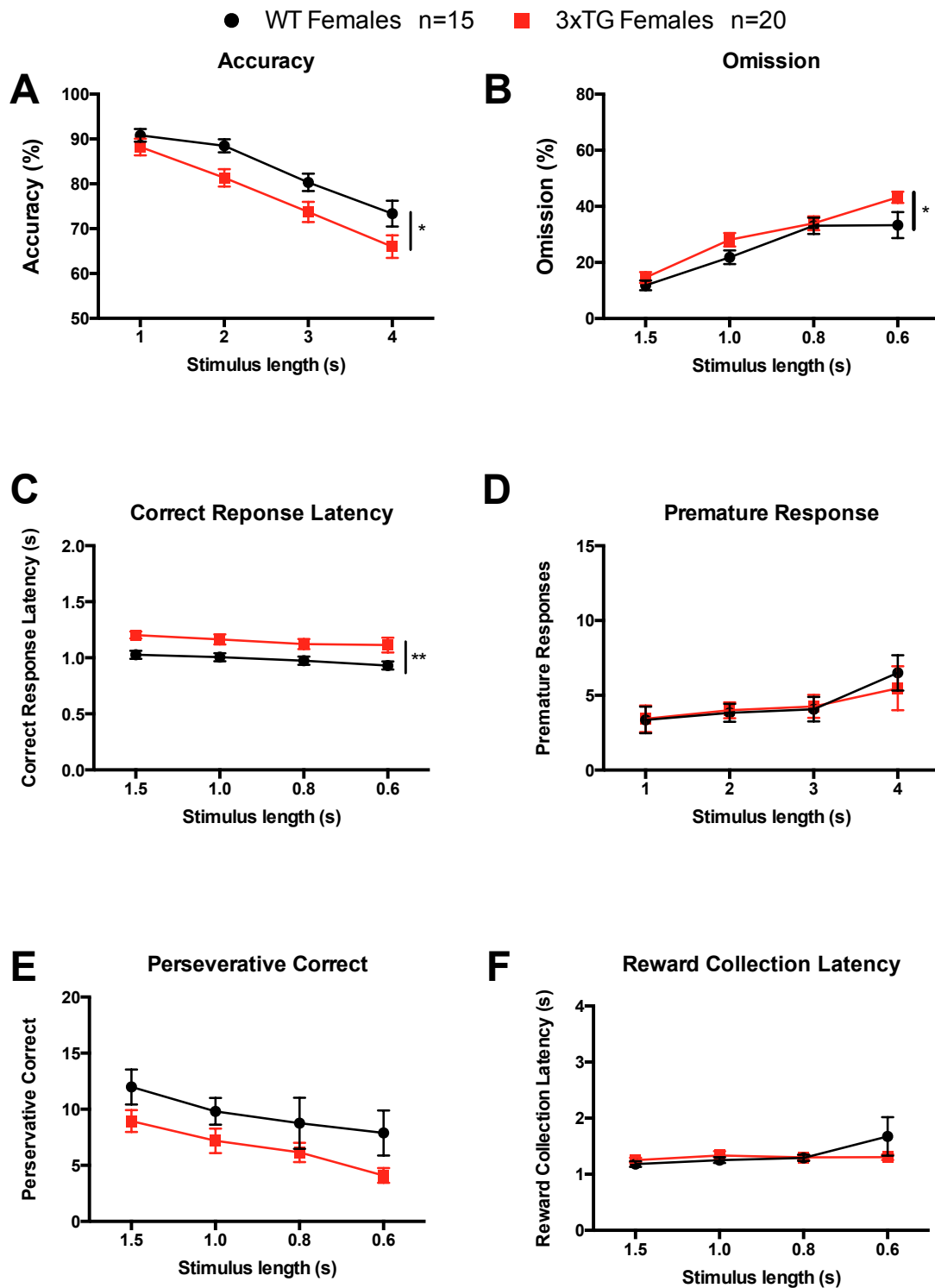


Figure 16. Performance and response measures of the combined data for 7 month-old 3xTG Females at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG females n=20, WT females n=15, data are mean \pm SEM, * p <0.05 and ** p <0.01)

data revealed that the 3xTG females had deficits in omissions compared to controls (**Figure 16B**; RM-ANOVA, main effect of genotype, $F_{(1,32)} = 4.552$, $p = 0.0406$; main effect of stimulus duration, $F_{(3,96)} = 43.13$, $p < 0.0001$; no interaction effect, $F_{(3,96)} = 1.413$, $p = 0.2437$). Differences were also observed between genotypes in correct response latency (**Figure 16C**; RM-ANOVA, main effect of genotype, $F_{(1,32)} = 10.87$, $p = 0.0024$; main effect of stimulus duration, $F_{(3,96)} = 2.868$, $p = 0.0405$; no interaction effect, $F_{(3,96)} = 0.1061$, $p = 0.9563$). The combined data revealed no differences between females 3xTG and control mice in premature responses (**Figure 16D**; RM-ANOVA, no effect of genotype, $F_{(1,32)} = 0.0202$, $p = 0.8880$; main effect of stimulus duration, $F_{(3,96)} = 4.567$, $p = 0.0049$; no interaction effect, $F_{(3,96)} = 0.3085$, $p = 0.8192$), perseverative correct (**Figure 16E**; RM-ANOVA, no effect of genotype, $F_{(1,32)} = 4.868$, $p = 0.0547$; main effect of stimulus duration, $F_{(3,96)} = 7.045$, $p = 0.0002$; no interaction effect, $F_{(3,96)} = 0.0609$, $p = 0.9802$), or reward collection latency (**Figure 16F**; RM-ANOVA, no effect of genotype, $F_{(1,32)} = 0.3668$, $p = 0.5490$; no effect of stimulus duration, $F_{(3,96)} = 2.188$, $p = 0.0945$; no interaction effect, $F_{(3,96)} = 1.831$, $p = 0.1467$).

At 10 months of age, combined data revealed female 3xTG mice to have significantly decreased accuracy compared to controls (**Figure 17A**; RM-ANOVA, main effect of genotype, $F_{(1,33)} = 5.324$, $p = 0.0275$; main effect of stimulus duration, $F_{(3,99)} = 63.86$, $p < 0.0001$; no interaction effect, $F_{(3,99)} = 1.069$, $p = 0.3659$). However, no difference was observed in omissions (**Figure 17B**; RM-ANOVA, no effect of genotype, $F_{(1,33)} = 0.0313$, $p = 0.8606$; main effect of stimulus duration, $F_{(3,99)} = 52.14$, $p < 0.0001$; no interaction effect, $F_{(3,99)} = 1.039$, $p = 0.3788$). The combined data also demonstrated that transgenic females have an increased correct response latency compared to age-matched controls

(**Figure 17C**; RM-ANOVA, main effect of genotype, $F_{(1,33)} = 9.202$, $p = 0.0047$; main effect of stimulus duration, $F_{(3,99)} = 4.156$, $p = 0.0081$; no interaction effect, $F_{(3,99)} = 0.9700$, $p = 0.4101$). The 10 month-old females did not display any differences in premature responses (**Figure 17D**; RM-ANOVA, no effect of genotype, $F_{(1,33)} = 0.0873$, $p = 0.7695$; no effect of stimulus duration, $F_{(3,99)} = 1.883$, $p = 0.1374$; no interaction effect, $F_{(3,99)} = 0.1473$, $p = 0.9312$), perseverative correct, although there was a tendency for the transgenic mice to have lower responses compared to controls, (**Figure 17E**; RM-ANOVA, no effect of genotype, $F_{(1,33)} = 3.663$, $p = 0.0643$; main effect of stimulus duration, $F_{(3,99)} = 8.001$, $p < 0.0001$; no interaction effect, $F_{(3,99)} = 2.262$, $p = 0.0860$), or reward collection latency (**Figure 17F**; RM-ANOVA, no effect of genotype, $F_{(1,33)} = 1.385$, $p = 0.2477$; no effect of stimulus duration, $F_{(3,99)} = 0.4324$, $p = 0.7303$; no interaction effect, $F_{(3,99)} = 1.078$, $p = 0.3621$).

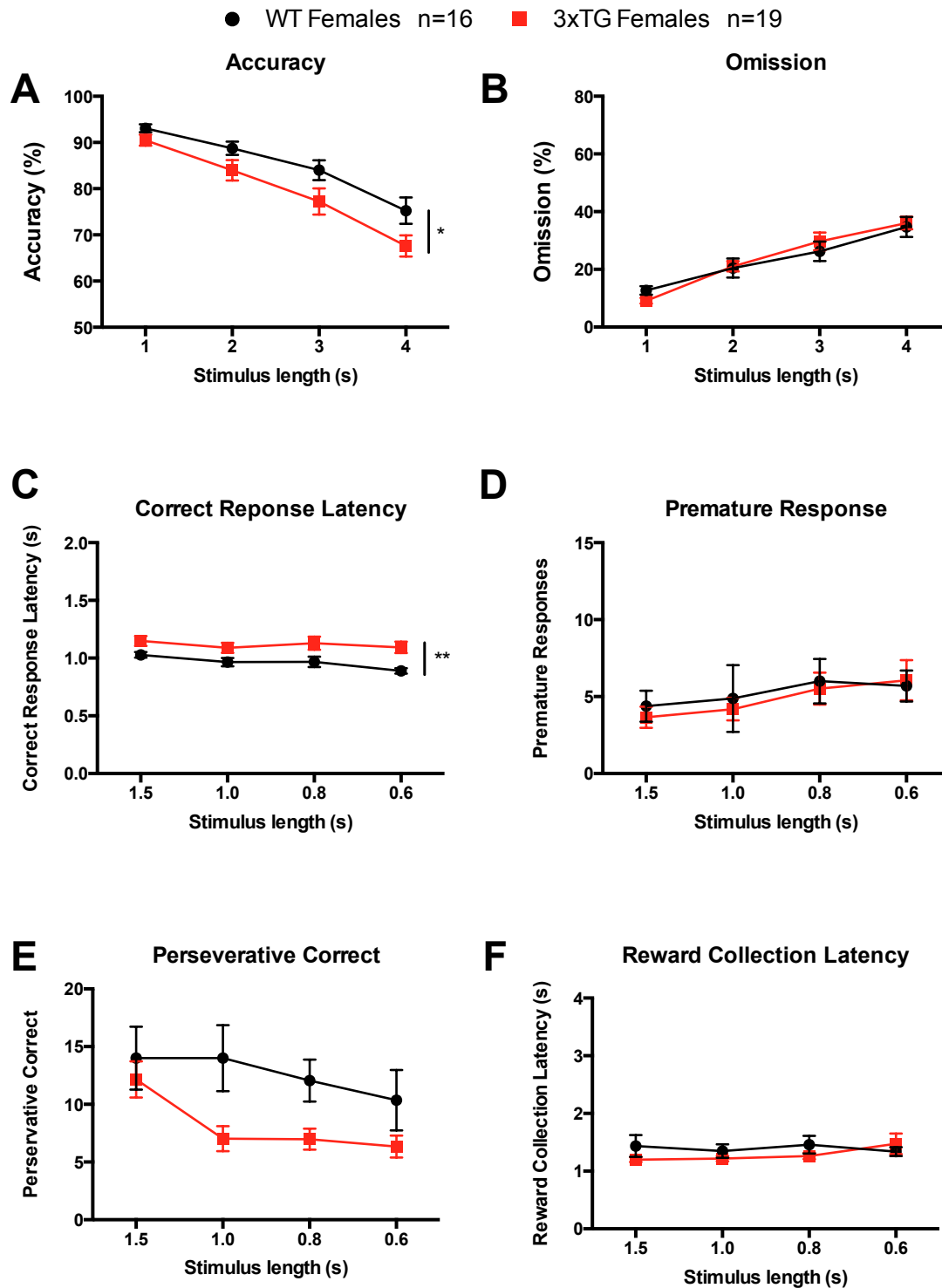


Figure 17. Performance and response measures of the combined data for 10 month-old 3xTG Females at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (3xTG females n=19, WT females n=16, data are mean \pm SEM, * p <0.05 and ** p <0.01)

3.5 Attention Capacity of Male 5xFAD mice

Several AD mouse lines have been generated presenting distinct deficits at different ages (Webster et al., 2014). Whether distinct AD mice may present similar phenotypes that could be predictable for future interventions needs to be clarified. We therefore obtained data for attentional performance in a second AD mouse line which presents fast developing amyloidosis due to combined mutations in APP and PSEN1, the 5xFAD line (Oakley et al., 2006). We chose to test this mouse line longitudinally at 4.5, 7 and 10 months of age on the 5-CSRTT based on data from previous experiments that have shown the progression of AD-like pathology (Oakley et al., 2006). In addition, testing at the same ages as the 3xTG mice allowed us to determine to what extent there were any age-dependent differences between the mouse lines.

The 4.5 month-old 5xFAD male mice did not show any difference in accuracy compared to controls during the first probe trials (**Figure 18A**; RM-ANOVA, no effect of genotype, $F_{(1,19)} = 0.2634$, $p = 0.6137$; main effect of stimulus duration, $F_{(3,57)} = 27.06$, $p < 0.0001$; no interaction effect, $F_{(3,57)} = 0.8136$, $p = 0.4917$). In addition, performance did not differ between the 5xFAD males and controls in omissions (**Figure 18B**; RM-ANOVA, no effect of genotype, $F_{(1,19)} = 0.4977$, $p = 0.4891$; main effect of stimulus duration, $F_{(3,57)} = 54.79$, $p < 0.0001$; no interaction effect, $F_{(3,57)} = 0.5362$, $p = 0.6594$), correct response latency (**Figure 18C**; RM-ANOVA, no effect of genotype, $F_{(1,19)} = 3.832$, $p = 0.0651$; no effect of stimulus duration, $F_{(3,57)} = 2.645$, $p = 0.0577$; no interaction effect, $F_{(3,57)} = 0.1085$, $p = 0.9548$), premature responses (**Figure 18D**; RM-ANOVA, no effect of genotype, $F_{(1,19)} = 0.0606$, $p = 0.8082$; main effect of stimulus duration, $F_{(3,57)} = 2.918$, $p = 0.0481$; no interaction effect, $F_{(3,57)} = 6.003$, $p = 0.0013$), perseverative correct

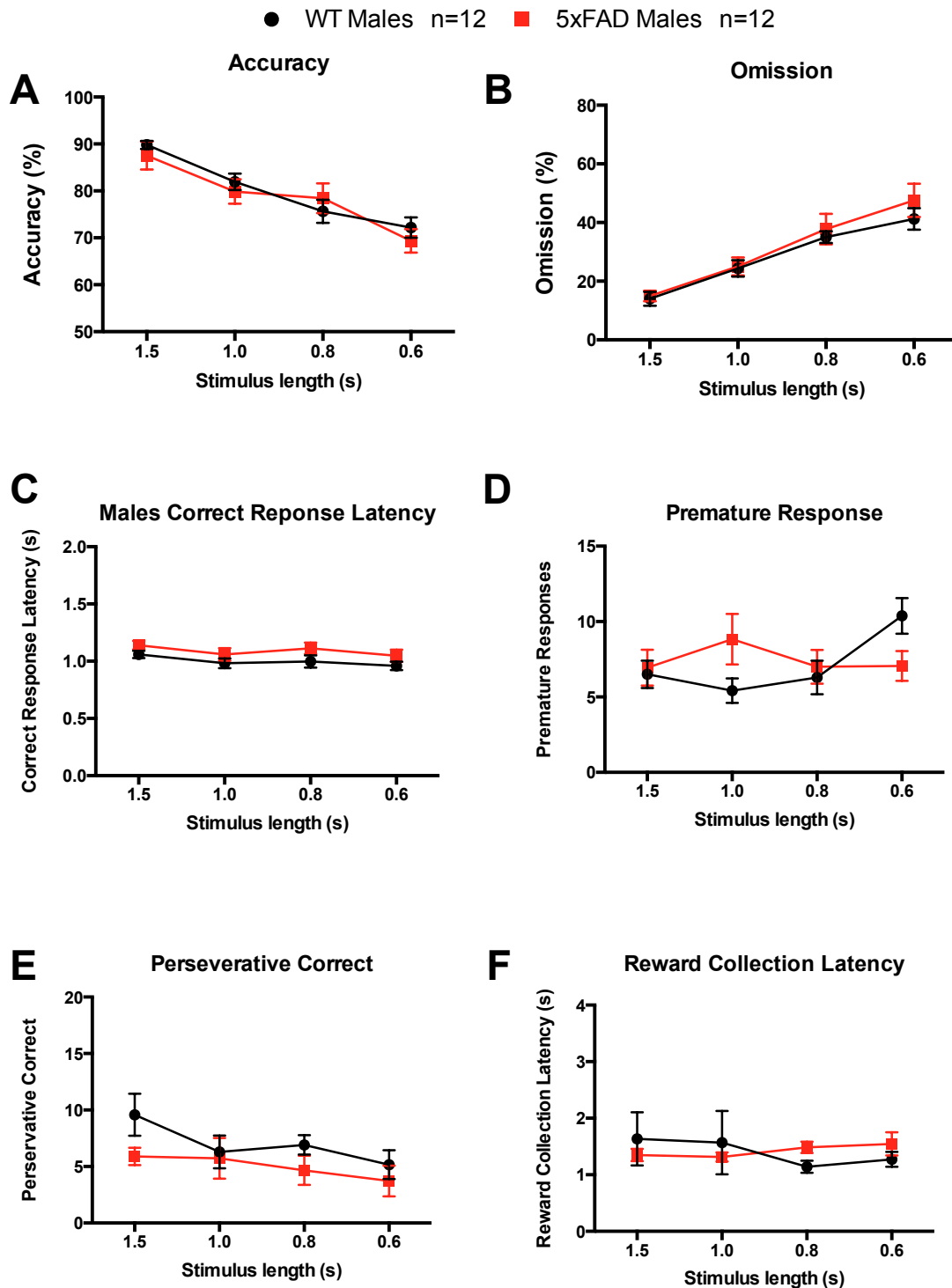


Figure 18. Performance and response measures for 4.5 month-old 5xFAD Males at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD males n=12, WT males n=12, data are mean \pm SEM)

responses (**Figure 18E**; RM-ANOVA, no effect of genotype, $F_{(1,19)} = 1.749$, $p = 0.2017$; main effect of stimulus duration, $F_{(3,57)} = 3.097$, $p = 0.0339$; no interaction effect, $F_{(3,57)} = 0.7475$, $p = 0.5283$), or reward collection latency (**Figure 18F**; RM-ANOVA, no effect of genotype, $F_{(1,19)} = 0.0077$, $p = 0.9310$; no effect of stimulus duration, $F_{(3,57)} = 0.1122$, $p = 0.9527$; no interaction effect, $F_{(3,57)} = 0.05497$, $p = 0.6504$).

Similarly, at 7 months, performance of 5xFAD males did not differ from that of controls in accuracy (**Figure 19A**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 2.403$, $p = 0.1360$; main effect of stimulus duration, $F_{(3,63)} = 28.34$, $p < 0.0001$; main interaction effect, $F_{(3,63)} = 3.157$, $p = 0.0308$), or omissions (**Figure 19B**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.6592$, $p = 0.4260$; main effect of stimulus duration, $F_{(3,63)} = 29.56$, $p < 0.0001$; no interaction effect, $F_{(3,63)} = 1.304$, $p = 0.2811$). Although no significant differences were observed between genotypes in correct response latency (**Figure 19C**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 4.807$, $p = 0.0562$; no effect of stimulus duration, $F_{(3,63)} = 1.333$, $p = 0.2718$; no interaction effect, $F_{(3,63)} = 0.3872$, $p = 0.7626$), there is a strong tendency for 5xFAD males to take longer to respond. In addition, no differences were observed between genotypes in premature responses (**Figure 19D**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.0034$, $p = 0.9544$; no effect of stimulus duration, $F_{(3,63)} = 2.260$, $p = 0.0901$; no interaction effect, $F_{(3,63)} = 2.823$, $p = 0.0558$), or perseverative correct (**Figure 19E**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 2.492$, $p = 0.1293$; main effect of stimulus duration, $F_{(3,63)} = 3.090$, $p = 0.0333$; no interaction effect, $F_{(3,63)} = 0.9327$, $p = 0.4302$). The male 5xFAD mice did have higher reward collection latencies compared to controls (**Figure 19F**; RM-ANOVA, main effect of

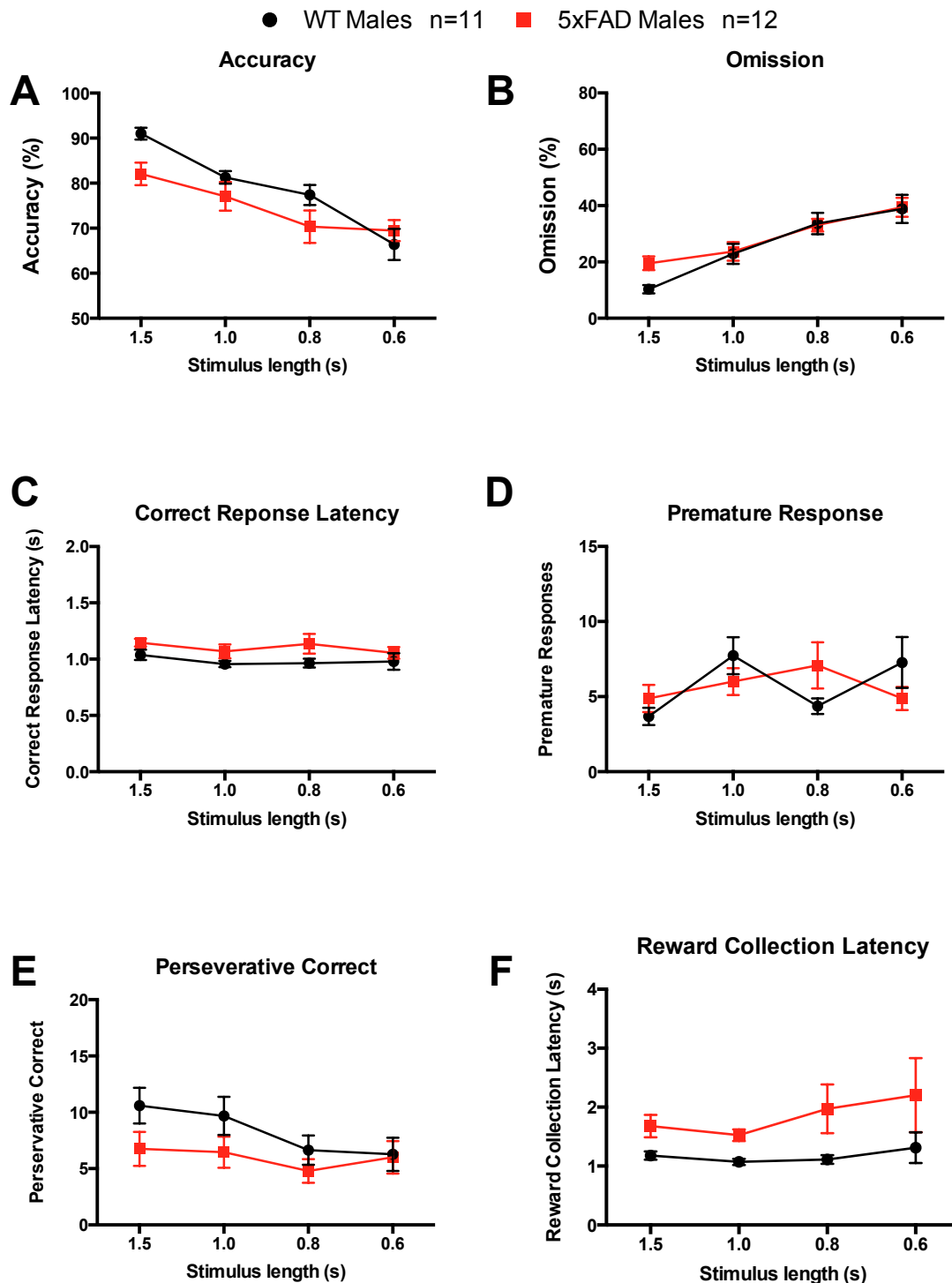


Figure 19. Performance and response measures for 7 month-old 5xFAD Males at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD males n=12, WT males n=11, data are mean \pm SEM)

genotype, $F_{(1,21)} = 4.671$, $p = 0.0424$; no effect of stimulus duration, $F_{(3,63)} = 1.290$, $p = 0.2856$; no interaction effect, $F_{(3,63)} = 0.4530$, $p = 0.7161$).

Interestingly, at 10 months of age, 5xFAD male mice revealed decreased accuracy, the main measure of attention in the 5-CSRTT, with both a genotype and interaction difference (**Figure 20A**; RM-ANOVA, main effect of genotype, $F_{(1,22)} = 5.824$, $p = 0.0293$; main effect of stimulus duration, $F_{(3,66)} = 54.53$, $p < 0.0001$; main interaction effect, $F_{(3,66)} = 2.879$, $p = 0.0425$). *Post-hoc* analysis demonstrated significant differences at both the 0.8s and 0.6s stimulus duration between genotypes (**Fig. 20A**). No differences in omission were observed between transgenic and wild-type males (**Figure 20B**; RM-ANOVA, no effect of genotype, $F_{(1,22)} = 3.030$, $p = 0.0957$; main effect of stimulus duration, $F_{(3,66)} = 24.59$, $p < 0.0001$; no interaction effect, $F_{(3,66)} = 1.165$, $p = 0.3296$). In addition, 10 month-old 5xFAD males had a significantly higher correct response latency compared to age-matched controls (**Figure 20C**; RM-ANOVA, main effect of genotype, $F_{(1,22)} = 15.38$, $p = 0.0007$; main effect of stimulus duration, $F_{(3,66)} = 23.87$, $p < 0.0001$; no interaction effect, $F_{(3,66)} = 1.618$, $p = 0.1936$), but showed no difference in premature responses (**Figure 20D**; RM-ANOVA, no effect of genotype, $F_{(1,22)} = 0.4828$, $p = 0.4944$; main effect of stimulus duration, $F_{(3,66)} = 4.437$, $p = 0.0067$; no interaction effect, $F_{(3,66)} = 1.282$, $p = 0.2879$). In contrast, 5xFAD males demonstrated a difference from control mice in both perseverative correct responses (**Figure 20E**; RM-ANOVA, main effect of genotype, $F_{(1,22)} = 11.66$, $p = 0.0025$; main effect of stimulus duration, $F_{(3,66)} = 10.18$, $p < 0.0001$; no interaction effect, $F_{(3,66)} = 2.223$, $p = 0.0937$), and reward collection latency (**Figure 20F**; RM-ANOVA, main effect of genotype, $F_{(1,22)} = 34.34$, $p < 0.0001$; no effect

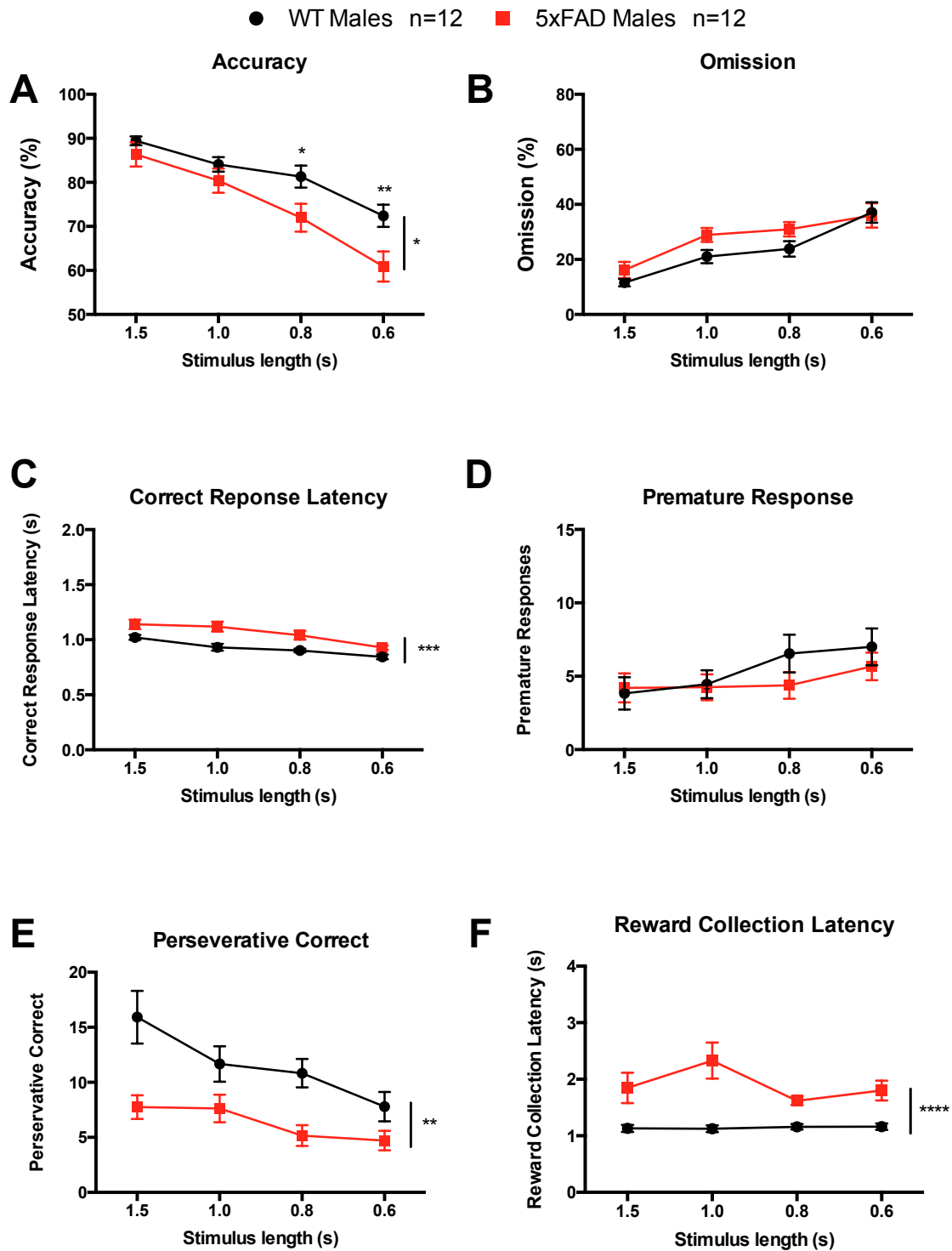


Figure 20. Performance and response measures for 10 month-old 5xFAD Males at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD males n=12, WT males n=12, data are mean \pm SEM, * p <0.05, ** p <0.01, *** p <0.001 and **** p <0.0001)

of stimulus duration, $F_{(3,66)} = 1.617, p = 0.1937$; no interaction effect, $F_{(3,66)} = 1.954, p = 0.1294$) compared to controls.

3.6 Performance of 5xFAD Female Mice

We also investigated whether 5xFAD female mice display any deficits in the 5-CSRTT compared to controls. 4.5 month-old 5xFAD females did not show a difference in accuracy compared to wild-type controls (**Figure 21A**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.3098, p = 0.5837$; main effect of stimulus duration, $F_{(3,63)} = 36.61, p < 0.0001$; no interaction effect, $F_{(3,63)} = 1.187, p = 0.3221$). Likewise, no difference was observed between the genotypes in omissions (**Figure 21B**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.0742, p = 0.7880$; main effect of stimulus duration, $F_{(3,63)} = 42.37, p < 0.0001$; no interaction effect, $F_{(3,63)} = 2.264, p = 0.0897$), correct response latency (**Figure 21C**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.7868, p = 0.3851$; main effect of stimulus duration, $F_{(3,63)} = 12.57, p < 0.0001$; no interaction effect, $F_{(3,63)} = 0.7074, p = 0.5512$), premature responses (**Figure 21D**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.0122, p = 0.9131$; main effect of stimulus duration, $F_{(3,63)} = 4.332, p = 0.0077$; no interaction effect, $F_{(3,63)} = 0.01206, p = 0.9476$), perseverative correct (**Figure 21E**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 2.605, p = 0.1214$; main effect of stimulus duration, $F_{(3,63)} = 3.665, p = 0.0168$; no interaction effect, $F_{(3,63)} = 0.1445, p = 0.9328$), or reward collection latency (**Figure 21F**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 0.0005, p = 0.9819$; no effect of stimulus duration, $F_{(3,63)} = 1.700, p = 0.1760$; no interaction effect, $F_{(3,63)} = 0.1528, p = 0.9275$).

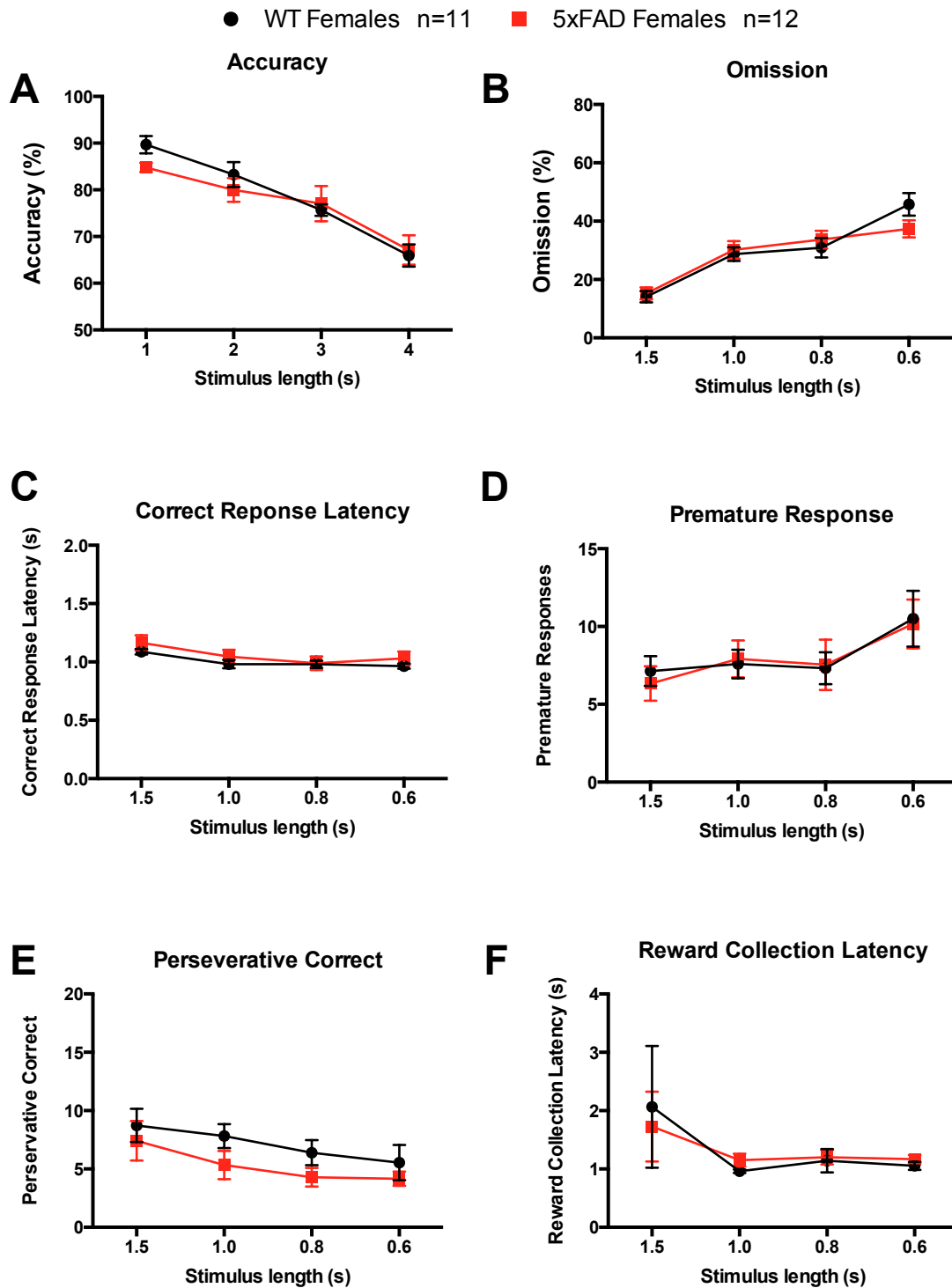


Figure 21. Performance and response measures for 4.5 month-old 5xFAD Females at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD females n=12, WT females n=11, data are mean \pm SEM)

The 7 month-old female 5xFAD mice did not reveal a difference in accuracy, albeit there was a tendency for worse performance by the transgenic mice (**Figure 22A**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 3.081$, $p = 0.0972$; main effect of stimulus duration, $F_{(3,51)} = 28.75$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 1.407$, $p = 0.2513$) or omissions (**Figure 22B**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 1.937$, $p = 0.1819$; main effect of stimulus duration, $F_{(3,51)} = 20.93$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 0.9139$, $p = 0.4408$). However, 5xFAD females did display an increased correct response latency compared to controls (**Figure 22C**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 11.37$, $p = 0.0036$; no effect of stimulus duration, $F_{(3,51)} = 1.248$, $p = 0.3021$; no interaction effect, $F_{(3,51)} = 1.788$, $p = 0.1611$), but no difference was observed in premature responses (**Figure 22D**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 0.5379$, $p = 0.4733$; no effect of stimulus duration, $F_{(3,51)} = 1.705$, $p = 0.1776$; no interaction effect, $F_{(3,51)} = 0.5160$, $p = 0.6732$). A difference was observed between genotypes for perseverative correct (**Figure 22E**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 6.266$, $p = 0.0228$; no effect of stimulus duration, $F_{(3,51)} = 2.183$, $p = 0.1014$; no interaction effect, $F_{(3,51)} = 0.5370$, $p = 0.6590$), as well as reward collection latency (**Figure 22F**; RM-ANOVA, main effect of genotype, $F_{(1,17)} = 5.645$, $p = 0.0295$; main effect of stimulus duration, $F_{(3,51)} = 3.886$, $p = 0.0141$; no interaction effect, $F_{(3,51)} = 0.9660$, $p = 0.4160$).

At 10 months of age, female 5xFAD mice showed a significant decrease in accuracy compared to controls (**Figure 23A**; RM-ANOVA, main effect of genotype, $F_{(1,14)} = 5.434$, $p = 0.0352$; main effect of stimulus duration, $F_{(3,42)} = 23.85$, $p < 0.0001$; no interaction effect, $F_{(3,42)} = 1.530$, $p = 0.2208$). However, no difference was observed

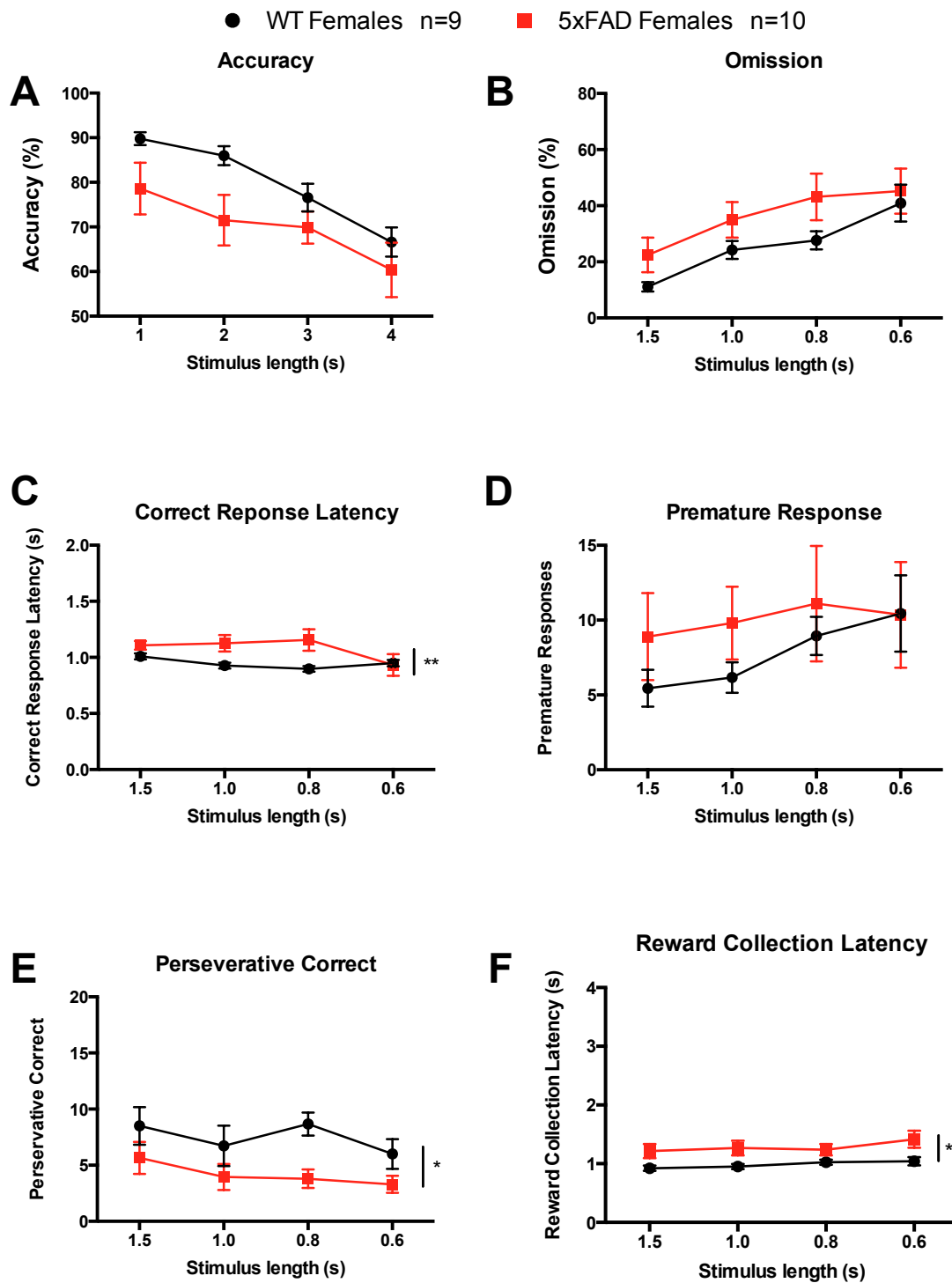


Figure 22. Performance and response measures for 7 month-old 5xFAD Females at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD females n=10, WT females n=9, data are mean \pm SEM, * p <0.05 and ** p <0.01)

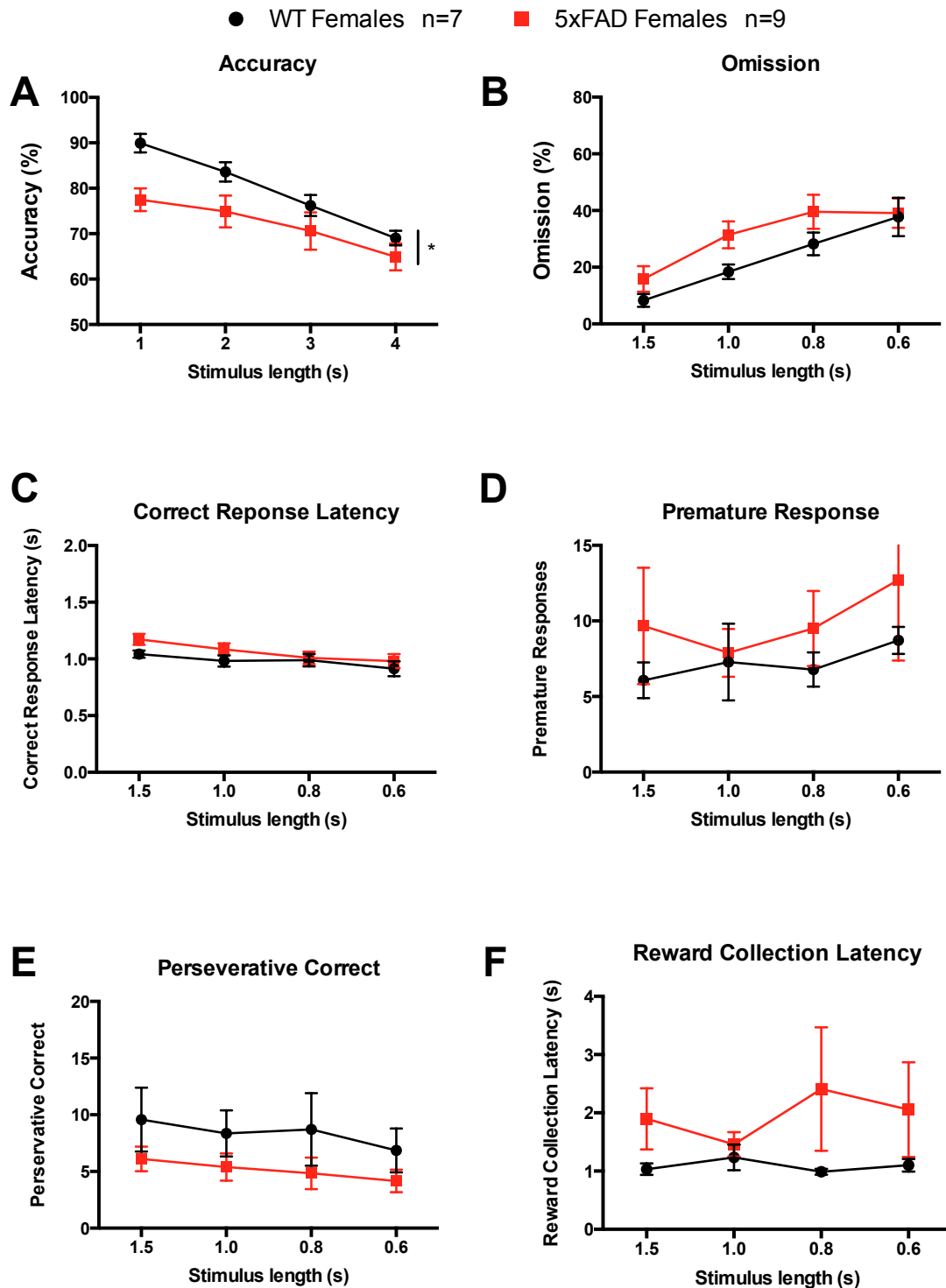


Figure 23. Performance and response measures for 10 month-old 5xFAD Females at the University of Western Ontario during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD females n=9, WT females n=7, data are mean \pm SEM)

between genotypes in omissions (**Figure 23B**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 1.987$, $p = 0.1805$; main effect of stimulus duration, $F_{(3,42)} = 29.56$, $p < 0.0001$; no interaction effect, $F_{(3,42)} = 1.465$, $p = 0.2379$), correct response latency (**Figure 23C**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 1.639$, $p = 0.2213$; main effect of stimulus duration, $F_{(3,42)} = 6.632$, $p = 0.0009$; no interaction effect, $F_{(3,42)} = 0.8247$, $p = 0.4877$), premature responses (**Figure 23D**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 0.6063$, $p = 0.4492$; no effect of stimulus duration, $F_{(3,42)} = 0.9553$, $p = 0.4227$; no interaction effect, $F_{(3,42)} = 0.2637$, $p = 0.8512$), perseverative correct responses (**Figure 23E**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 2.369$, $p = 0.1461$; no effect of stimulus duration, $F_{(3,42)} = 1.194$, $p = 0.3238$; no interaction effect, $F_{(3,42)} = 0.0909$, $p = 0.9646$), or reward collection latency (**Figure 23F**; RM-ANOVA, no effect of genotype, $F_{(1,14)} = 1.875$, $p = 0.1925$; no effect of stimulus duration, $F_{(3,42)} = 2257$, $p = 0.8780$; no interaction effect, $F_{(3,42)} = 0.6030$, $p = 0.6167$).

3.7 Reproducibility of 5xFAD Data Between Sites

The 5xFAD mouse line was also run simultaneously at site 1 and site 2 in order to determine whether the 5-CSRTT data are reproducible. Again, accuracy from the 10 month-old mice was analysed because this is where we observed the largest difference between genotypes. Male wild-type mice displayed no difference in accuracy between sites (**Figure 24A**; RM-ANOVA, no effect of genotype, $F_{(1,20)} = 1.191$, $p = 0.2881$; main effect of stimulus duration, $F_{(3,60)} = 36.22$, $p < 0.0001$; no interaction effect, $F_{(3,60)} = 0.2995$, $p = 0.8256$), nor did the 5xFAD mice (**Figure 24B**; RM-ANOVA, no effect of genotype, $F_{(1,21)} = 1.133$, $p = 0.2992$; main effect of stimulus duration, $F_{(3,63)} = 77.10$, $p <$

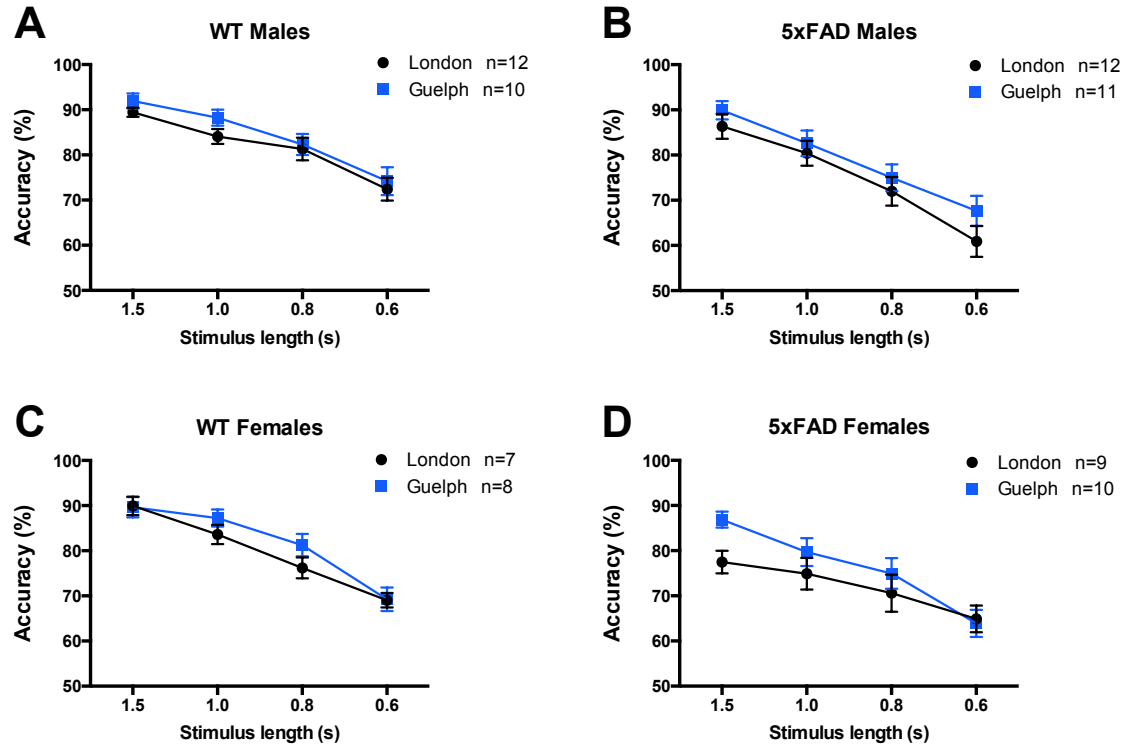


Figure 24. Comparison of the performance and response measures of 10 month-old 5xFAD and Wild-type Males and Females tested at the University of Western Ontario (London) and at the University of Guelph (Guelph) during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy for WT males. **B**, Mean accuracy for 5xFAD males from both sites. **C**, Mean accuracy for WT females. **D**, Mean accuracy for 5xFAD females. (Data are mean \pm SEM).

0.0001; no interaction effect, $F_{(3,63)} = 0.7266$, $p = 0.5399$). Likewise, 10 month-old wild-type females did not exhibit a difference in accuracy when compared between the two sites (**Figure 24C**; RM-ANOVA, no effect of genotype, $F_{(1,13)} = 0.8213$, $p = 0.3785$; main effect of stimulus duration, $F_{(3,39)} = 57.62$, $p < 0.0001$; no interaction effect, $F_{(3,39)} = 1.216$, $p = 0.3169$). There was also no difference observed in the performance of the two groups of 5xFAD females between sites (**Figure 24D**; RM-ANOVA, no effect of genotype, $F_{(1,17)} = 1.826$, $p = 0.1943$; main effect of stimulus duration, $F_{(3,51)} = 19.82$, $p < 0.0001$; no interaction effect, $F_{(3,51)} = 1.546$, $p = 0.2094$). The comparison of data for many of the other relevant parameters also revealed very little differences between sites (not shown). This suggests that data for the 5xFAD mouse line are also reproducible between sites 1 and 2. We obtained data from Dr. Winters' group and combined the analysis to increase statistical power and define the most robust phenotypes.

Attention capacity of male 5xFAD mice (combining the data from both sites) was analyzed. There was no difference in accuracy between 4.5 month-old 5xFAD males and controls (**Figure 25A**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 0.0001$, $p = 0.9968$; main effect of stimulus duration, $F_{(3,111)} = 43.46$, $p < 0.0001$; no interaction effect, $F_{(3,111)} = 0.5811$, $p = 0.6286$). In addition, no difference in genotypes were observed in omissions (**Figure 25B**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 0.3821$, $p = 0.5403$; main effect of stimulus duration, $F_{(3,111)} = 81.32$, $p < 0.0001$; no interaction effect, $F_{(3,111)} = 1.268$, $p = 0.2889$), correct response latency (**Figure 25C**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 2.576$, $p = 0.1170$; main effect of stimulus duration, $F_{(3,111)} = 4.981$, $p = 0.0028$; no interaction effect, $F_{(3,111)} = 0.3271$, $p = 0.8058$), premature responses (**Figure 25D**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 0.1064$, $p = 0.7461$; main

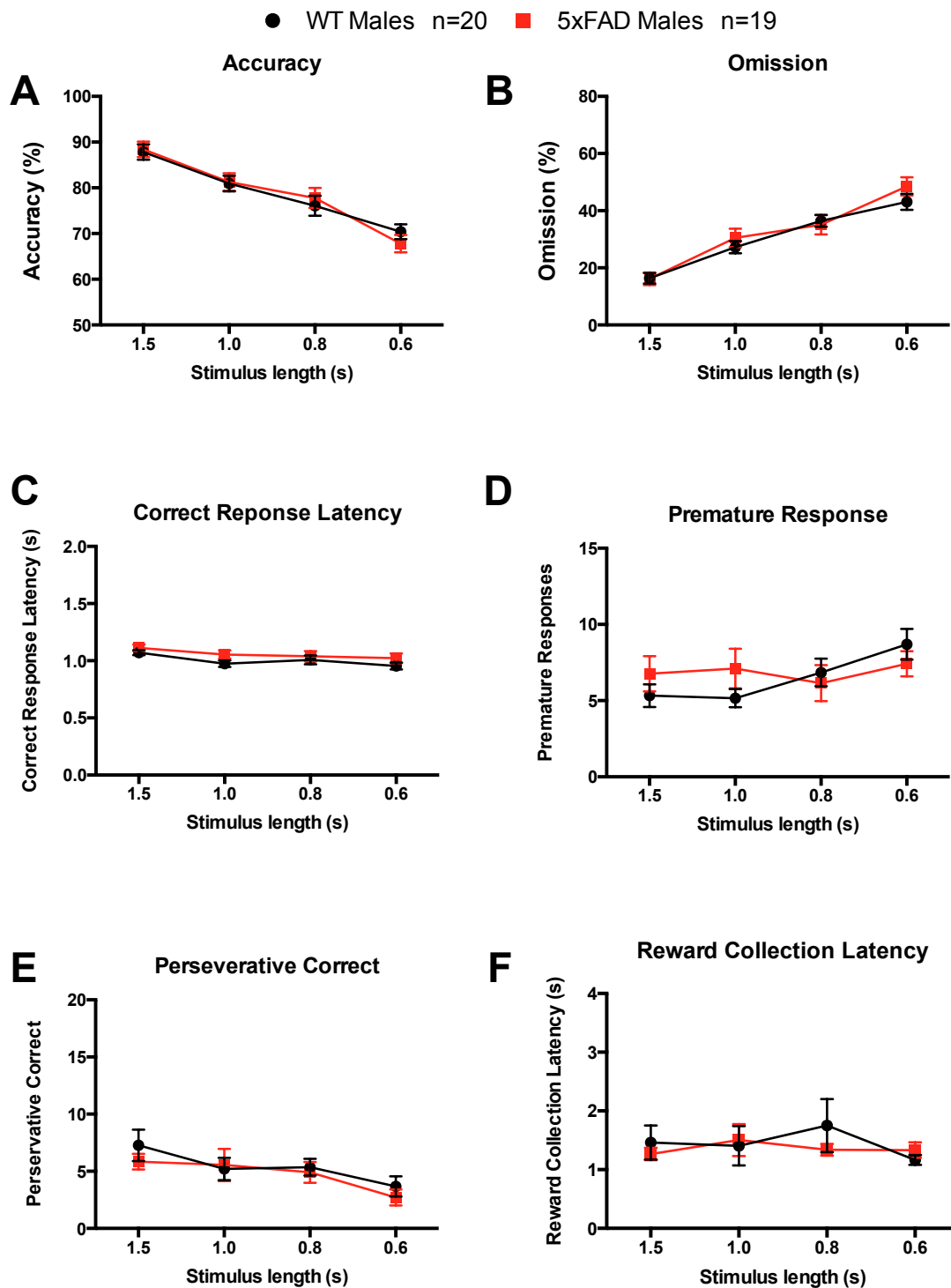


Figure 25. Performance and response measures of the combined data for 4.5 month-old 5xFAD Males at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD males n=19, WT males n=20, data are mean \pm SEM)

effect of stimulus duration, $F_{(3,111)} = 3.400, p = 0.0203$; no interaction effect, $F_{(3,111)} = 2.403, p = 0.0714$), perseverative correct responses (**Figure 25E**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 0.3285, p = 0.5700$; main effect of stimulus duration, $F_{(3,111)} = 7.363, p = 0.0002$; no interaction effect, $F_{(3,111)} = 0.5485, p = 0.6502$), or reward collection latency (**Figure 25F**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 0.2046, p = 0.6537$; main effect of stimulus duration, $F_{(3,111)} = 0.4887, p = 0.6908$; no interaction effect, $F_{(3,111)} = 0.5517, p = 0.6480$).

At 7 months, 5xFAD males from the combined data displayed a significant difference in accuracy compared to age-matched controls (**Figure 26A**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 0.5.658, p = 0.0219$; main effect of stimulus duration, $F_{(3,129)} = 63.72, p < 0.0001$; no interaction effect, $F_{(3,129)} = 0.4193, p = 0.7394$) but had no significant change in omissions (**Figure 26B**; RM-ANOVA, no effect of genotype, $F_{(1,43)} = 1.862, p = 0.1794$; main effect of stimulus duration, $F_{(3,129)} = 61.63, p < 0.0001$; no interaction effect, $F_{(3,129)} = 0.4579, p = 0.7122$). Combined data at 7 months did show male 5xFAD mice to have significant difference in correct response latency compared to controls (**Figure 26C**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 9.435, p = 0.0037$; main effect of stimulus duration, $F_{(3,129)} = 5.898, p = 0.0008$; no interaction effect, $F_{(3,129)} = 0.3240, p = 0.8080$). No difference between genotypes was observed in premature responses (**Figure 26D**; RM-ANOVA, no effect of genotype, $F_{(1,43)} = 0.4497, p = 0.5061$; main effect of stimulus duration, $F_{(3,129)} = 2.875, p = 0.0388$; no interaction effect, $F_{(3,129)} = 0.7579, p = 0.5197$). Also, combined data for 7 month-old 5xFAD males displayed a difference compared to controls in perseverative correct responses (**Figure 26E**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 10.54, p = 0.0023$; main effect of stimulus

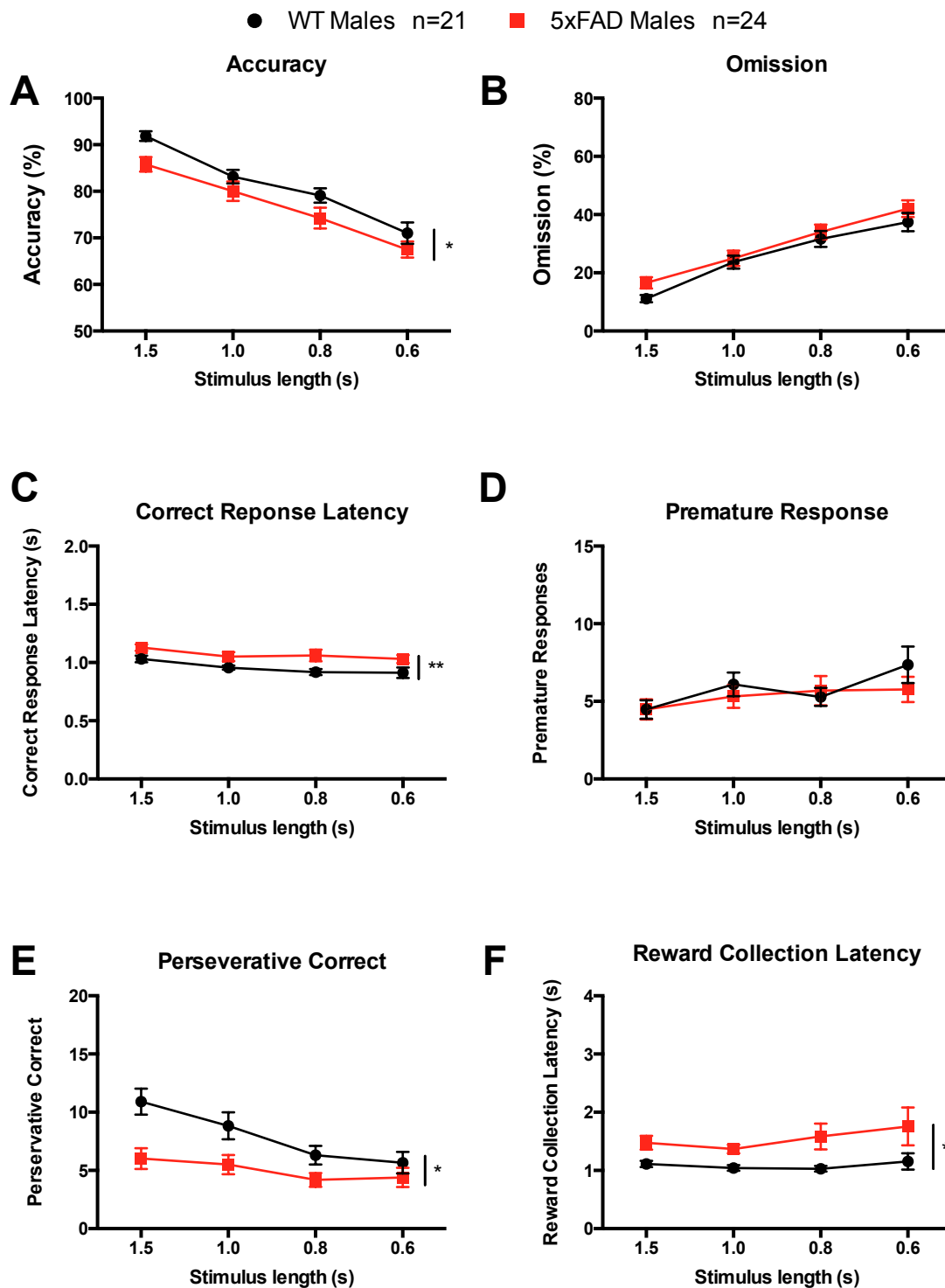


Figure 26. Performance and response measures of the combined data for 7 month-old 5xFAD Males at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD males n=24, WT males n=21, data are mean \pm SEM, * p <0.05 and ** p <0.01)

duration, $F_{(3,129)} = 9.837, p < 0.0001$; no interaction effect, $F_{(3,129)} = 2.265, p = 0.0841$), and decreased reward collection latency (**Figure 26F**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 6.657, p = 0.0134$; no effect of stimulus duration, $F_{(3,129)} = 1.354, p = 0.2599$; no interaction effect, $F_{(3,129)} = 0.5902, p = 0.6255$).

The combined data for 10 month-old 5xFAD males demonstrated increased deficits in accuracy with both a genotype and interaction difference (**Figure 27A**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 8.148, p = 0.0066$; main effect of stimulus duration, $F_{(3,129)} = 111.4, p < 0.0001$; main interaction effect, $F_{(3,129)} = 3.474, p = 0.0181$) and the *post-hoc* analysis revealed a significantly lower accuracy at both the 0.8s and 0.6s stimulus durations for the transgenic males compared to controls (**Fig. 27A**). In addition, significant differences were observed between genotypes in omissions (**Figure 27B**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 7.570, p = 0.0087$; main effect of stimulus duration, $F_{(3,129)} = 50.52, p < 0.0001$; no interaction effect, $F_{(3,129)} = 1.358, p = 0.2586$), and correct response latency (**Figure 27C**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 21.20, p < 0.0001$; main effect of stimulus duration, $F_{(3,129)} = 22.78, p < 0.0001$; main interaction effect, $F_{(3,129)} = 31.67, p = 0.0267$). The 10 month-old male 5xFAD mice did not show a difference in premature responses compared to controls in the combined data (**Figure 27D**; RM-ANOVA, no effect of genotype, $F_{(1,43)} = 0.0244, p = 0.8765$; main effect of stimulus duration, $F_{(3,129)} = 6.549, p = 0.0004$; no interaction effect, $F_{(3,129)} = 1.538, p = 0.2078$). However, compared to age-matched controls, the performance of 5xFAD males did differ for perseverative correct (**Figure 27E**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 11.19, p = 0.0017$; main effect of stimulus duration, $F_{(3,129)} = 11.93, p < 0.0001$; no interaction effect, $F_{(3,129)} = 0.3469, p = 0.7914$), and reward collection

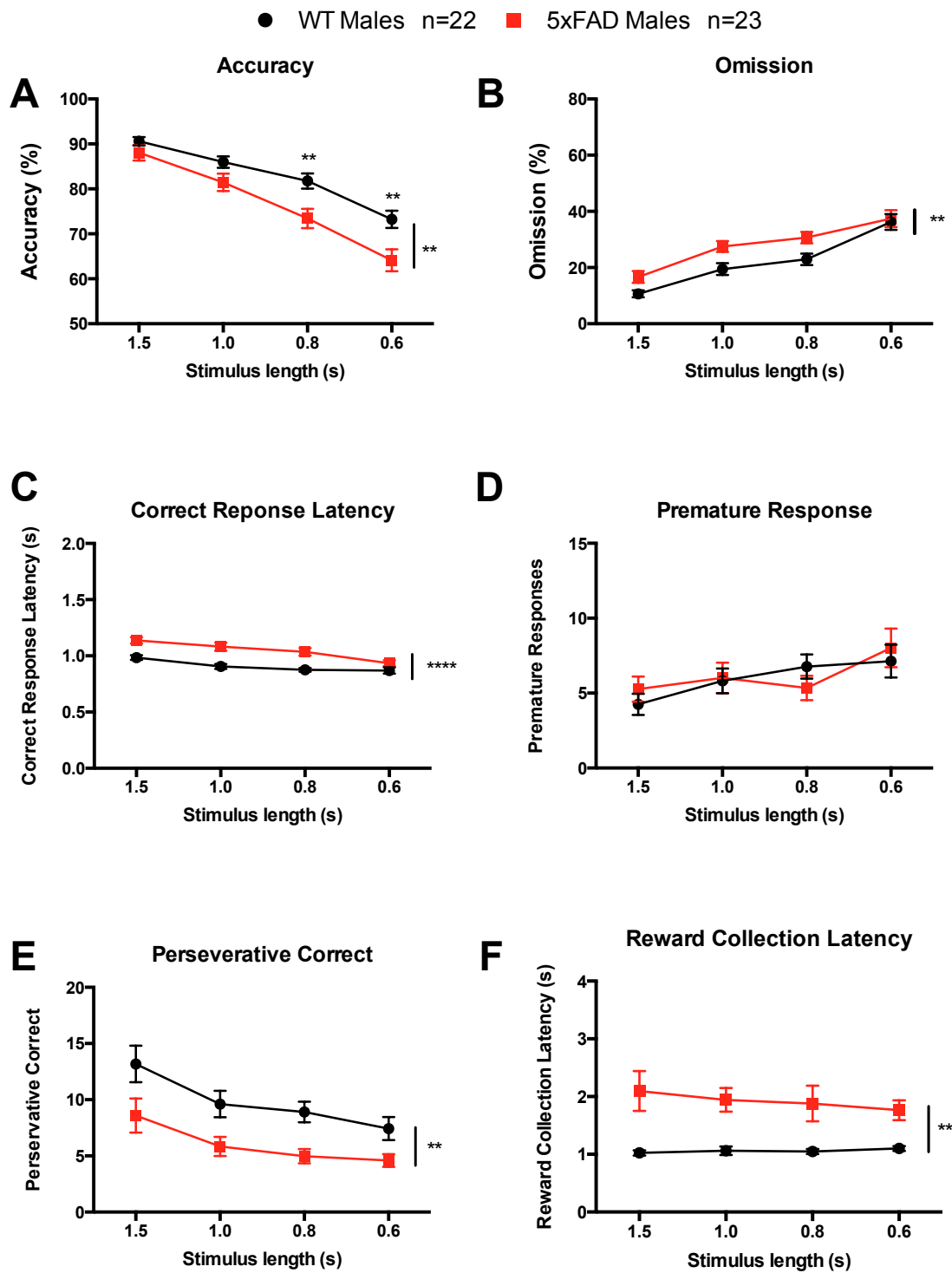


Figure 27. Performance and response measures of the combined data for 10 month-old 5xFAD Males at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD males n=23, WT males n=22, data are mean \pm SEM, **p<0.01 and ****p<0.0001)

latency (**Figure 27F**; RM-ANOVA, main effect of genotype, $F_{(1,43)} = 8.621$, $p = 0.0053$; no effect of stimulus duration, $F_{(3,129)} = 1.237$, $p = 0.2989$; no interaction effect, $F_{(3,129)} = 1.418$, $p = 0.2405$).

The performance of the 5xFAD females (combined data from both sites) was analyzed and revealed no differences in accuracy between the transgenic and wild-type controls at 4.5 months of age (**Figure 28A**; RM-ANOVA, no effect of genotype, $F_{(1,42)} = 0.5529$, $p = 0.4613$; main effect of stimulus duration, $F_{(3,126)} = 44.61$, $p < 0.0001$; no interaction effect, $F_{(3,126)} = 0.8749$, $p = 0.4561$). Similarly, no differences was observed between genotypes in omissions (**Figure 28B**; RM-ANOVA, no effect of genotype, $F_{(1,42)} = 0.0902$, $p = 0.7654$; main effect of stimulus duration, $F_{(3,126)} = 41.99$, $p < 0.0001$; no interaction effect, $F_{(3,126)} = 1.410$, $p = 0.2429$), correct response latency (**Figure 28C**; RM-ANOVA, no effect of genotype, $F_{(1,42)} = 0.4515$, $p = 0.5053$; main effect of stimulus duration, $F_{(3,126)} = 8.164$, $p < 0.0001$; no interaction effect, $F_{(3,126)} = 0.6658$, $p = 0.5746$), premature responses (**Figure 28D**; ; RM-ANOVA, no effect of genotype, $F_{(1,42)} = 0.4112$, $p = 0.5248$; no effect of stimulus duration, $F_{(3,126)} = 1.906$, $p = 0.1320$; no interaction effect, $F_{(3,126)} = 0.4807$, $p = 0.6963$), perseverative correct (**Figure 28E**; RM-ANOVA, no effect of genotype, $F_{(1,42)} = 0.6653$, $p = 0.4193$; main effect of stimulus duration, $F_{(3,126)} = 7.328$, $p = 0.0001$; no interaction effect, $F_{(3,126)} = 0.1218$, $p = 0.9471$), or reward collection latency (**Figure 28F**; RM-ANOVA, no effect of genotype, $F_{(1,42)} = 2.952$, $p = 0.0931$; no effect of stimulus duration, $F_{(3,126)} = 2.271$, $p = 0.0835$; no interaction effect, $F_{(3,126)} = 0.6320$, $p = 0.5957$).

The combined data for 7 month-old 5xFAD females revealed a significantly decreased accuracy compared to age-matched controls (**Figure 29A**; RM-ANOVA, main effect of

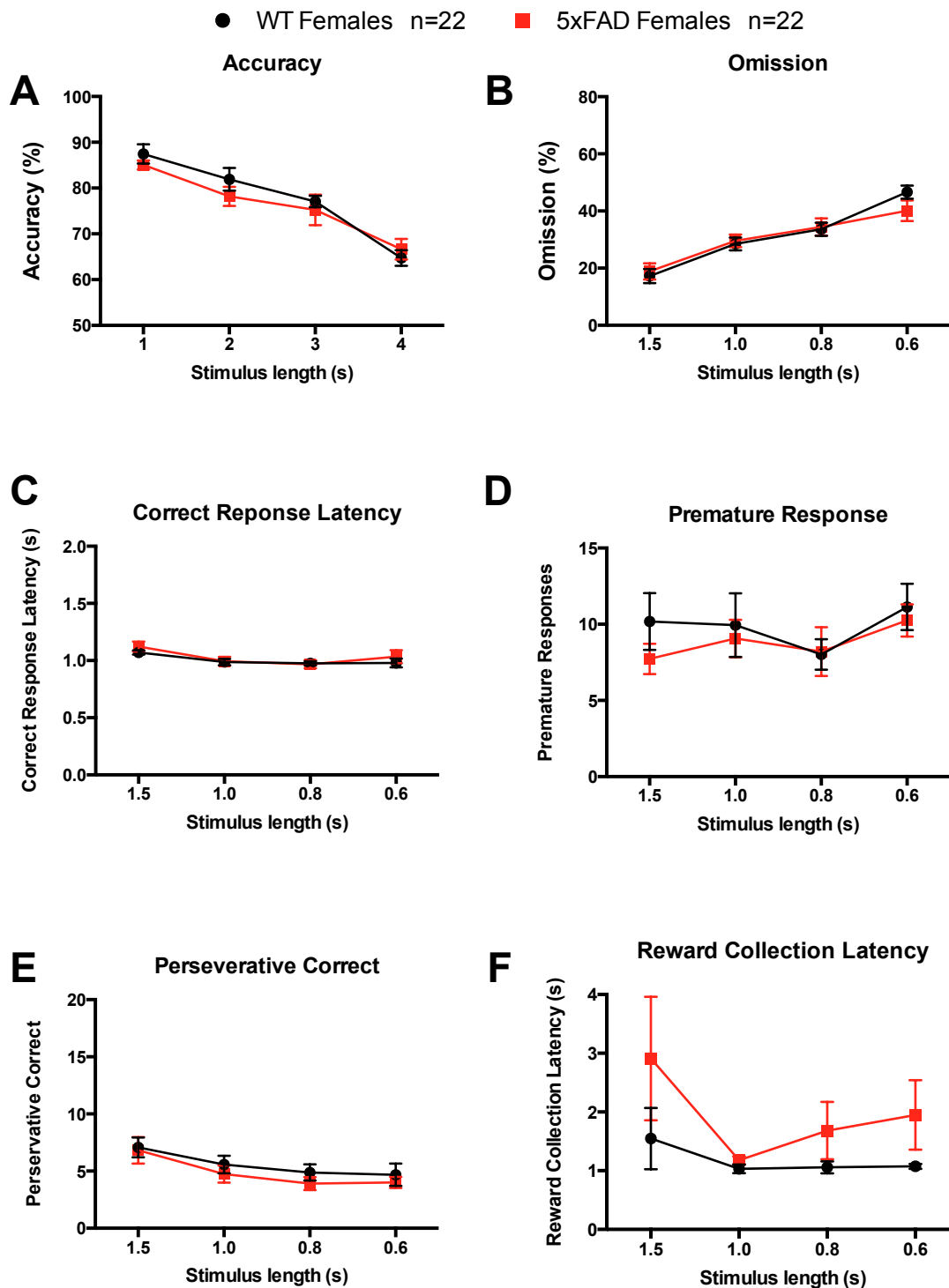


Figure 28. Performance and response measures of the combined data for 4.5 month-old 5xFAD Females at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD females n=22, WT females n=22, data are mean \pm SEM)

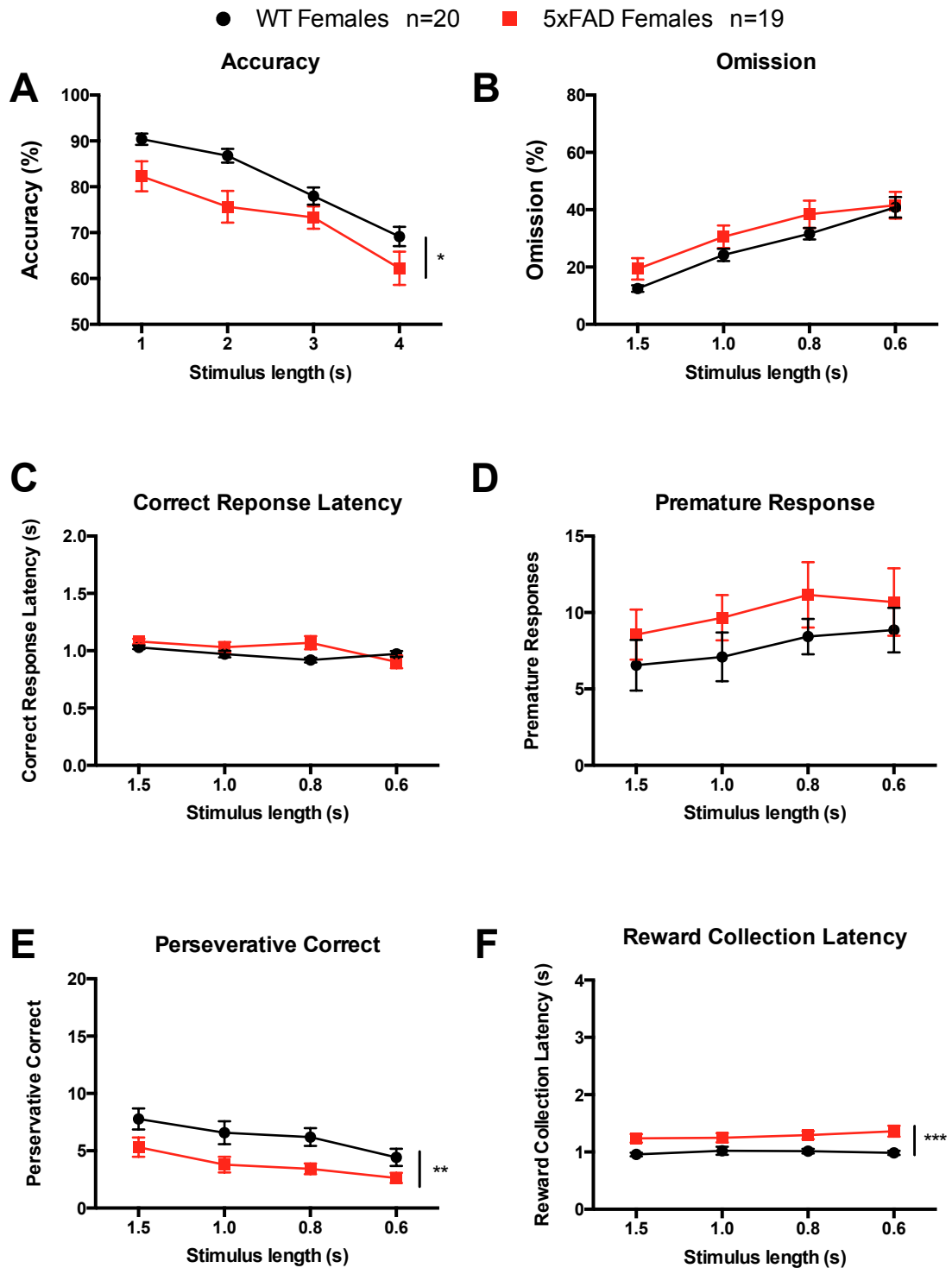


Figure 29. Performance and response measures of the combined data for 7 month-old 5xFAD Females at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD females n=19, WT females n=20, data are mean \pm SEM, * p <0.05, ** p <0.01 and *** p <0.001)

genotype, $F_{(1,37)} = 6.260, p = 0.0169$; main effect of stimulus duration, $F_{(3,111)} = 67.20, p < 0.0001$; no interaction effect, $F_{(3,111)} = 1.551, p = 0.2053$). There were no differences observed between genotypes in the combined data for omissions (**Figure 29B**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 1.692, p = 0.2014$; main effect of stimulus duration, $F_{(3,111)} = 46.59, p < 0.0001$; no interaction effect, $F_{(3,111)} = 0.8721, p = 0.4579$), correct response latency (**Figure 29C**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 2.690, p = 0.1094$; main effect of stimulus duration, $F_{(3,111)} = 3.767, p = 0.0128$; no interaction effect, $F_{(3,111)} = 3.306, p = 0.0529$), or premature responses (**Figure 29D**; RM-ANOVA, no effect of genotype, $F_{(1,37)} = 1.314, p = 0.2589$; no effect of stimulus duration, $F_{(3,111)} = 2.078, p = 0.1072$; no interaction effect, $F_{(3,111)} = 0.0798, p = 0.9709$). However, combined data for 7 month-old 5xFAD females did demonstrate significantly different perseverative correct responses (**Figure 29E**; RM-ANOVA, main effect of genotype, $F_{(1,37)} = 11.19, p = 0.0019$; main effect of stimulus duration, $F_{(3,111)} = 7.327, p = 0.0002$; no interaction effect, $F_{(3,111)} = 0.2583, p = 0.8552$), and reward collection latency compared to controls (**Figure 29F**; RM-ANOVA, main effect of genotype, $F_{(1,37)} = 15.12, p = 0.0004$; no effect of stimulus duration, $F_{(3,111)} = 1.204, p = 0.3117$; no interaction effect, $F_{(3,111)} = 1.085, p = 0.3585$).

Combined data for 10 month-old 5xFAD females displayed a difference in accuracy compared age-matched controls (**Figure 30A**; RM-ANOVA, main effect of genotype, $F_{(1,32)} = 9.415, p = 0.0044$; main effect of stimulus duration, $F_{(3,96)} = 56.68, p < 0.0001$; no interaction effect, $F_{(3,96)} = 0.4453, p = 0.7212$). In contrast, combined data for female 5xFAD mice suggest there was no difference in omission when compared to controls (**Figure 30B**; RM-ANOVA, no effect of genotype, $F_{(1,32)} = 2.111, p = 0.1560$; main effect

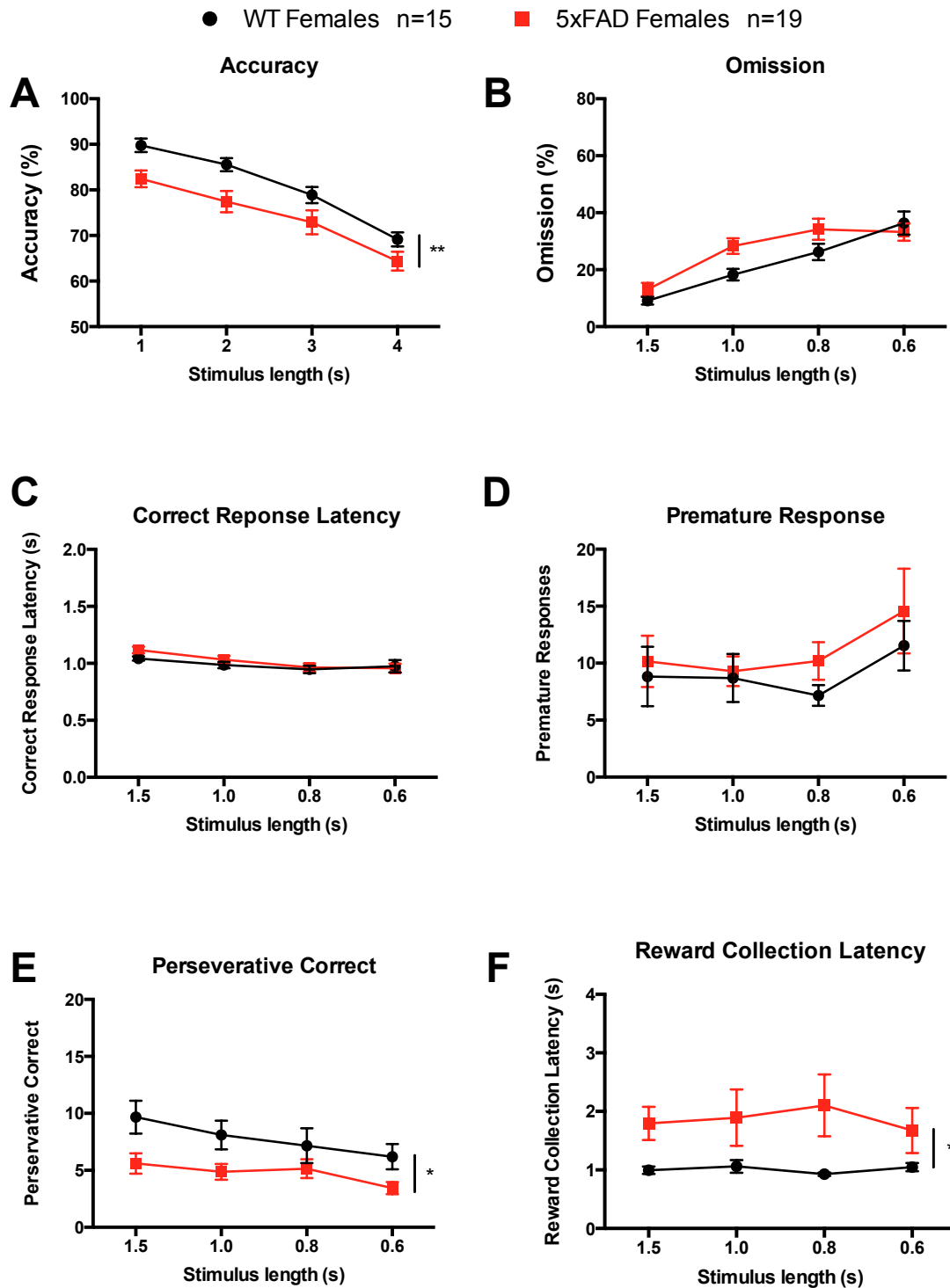


Figure 30. Performance and response measures of the combined data for 10 month-old 5xFAD Females at the University of Western Ontario and at the University of Guelph during the 5-CSRTT probe trials. Mice were subject to a series of probe trial schedules and the average values of the sessions are plotted. **A**, Mean accuracy. **B**, Mean omission. **C**, Mean correct response latency. **D**, Mean premature responses. **E**, Mean perseverative correct. **F**, Mean reward collection latency (5xFAD females n=19, WT females n=20, data are mean \pm SEM, *p<0.05 and **p<0.01)

of stimulus duration, $F_{(3,96)} = 46.05, p < 0.0001$; no interaction effect, $F_{(3,96)} = 3.649, p = 0.153$), correct response latency (**Figure 30C**; RM-ANOVA, no effect of genotype, $F_{(1,32)} = 0.6071, p = 0.4461$; main effect of stimulus duration, $F_{(3,96)} = 9.548, p < 0.0001$; no interaction effect, $F_{(3,96)} = 1.157, p = 0.3301$), or premature responses (**Figure 30D**; RM-ANOVA, no effect of genotype, $F_{(1,32)} = 0.5205, p = 0.4759$; main effect of stimulus duration, $F_{(3,96)} = 4.009, p = 0.0098$; no interaction effect, $F_{(3,96)} = 0.3724, p = 0.7731$). However, combined data did reveal genotype differences in perseverative correct responses (**Figure 30E**; RM-ANOVA, main effect of genotype, $F_{(1,32)} = 7.138, p = 0.0118$; main effect of stimulus duration, $F_{(3,96)} = 4.766, p = 0.0039$; no interaction effect, $F_{(3,96)} = 0.6510, p = 0.5842$), and reward collection latency (**Figure 30F**; RM-ANOVA, main effect of genotype, $F_{(1,32)} = 6.787, p = 0.0138$; no effect of stimulus duration, $F_{(3,96)} = 0.3265, p = 0.8062$; no interaction effect, $F_{(3,96)} = 0.6618, p = 0.5775$).

4 Discussion

4.1 Touchscreen systems diminish reproducibility problems associated with animal behavioural testing

Traditional mouse behavioural testing is usually slow, not amenable to high-throughput screening of a large number of animals and is far from being standardized. Unfortunately, conventional cognitive behavioural tests for mouse models are subject to extreme variation (Wahlsten et al., 2001, Wahlsten et al., 2003, Brown et al., 2009). The touchscreen-based 5-CSRTT in this study helped to improve these problems by allowing us to obtain replicable data between the two laboratories at site 1 and site 2.

The touchscreen systems gave us the opportunity to test a high number of animals daily and increase our sample size, thus improving statistical power of experiments. Research has shown that studies with a small number of subjects have a lower statistical power and hence a decreased chance of detecting a true effect (Button et al., 2013). In addition, research has revealed the average statistical power of studies in neuroscience is very low (Button et al., 2013). The data between site 1 and site 2 showed minimal variation and thus allowed us to combine the data within genotypes.

Traditional mouse behavioural testing also lacks translatable ability from the pre-clinical to clinical setting. For instance, multiple promising pharmacological treatments observed in animal models of AD failed to show similar results when tested in the clinic (Selkoe, 2012). The touchscreen systems utilized in this study are far more translatable to humans than other traditional mouse behavioural tests, such as the Morris Water maze or the Y-maze task. Based on the human CANTAB systems, the 5-CSRTT on the mouse touchscreen systems examine the same parameters as those observed in humans with AD

or MCI, hence increasing the translational potential for research in mouse models of AD (Bussey et al., 2008, Romberg et al., 2013a). The data obtained in this study help to provide an important baseline for the onset and progression of attention deficits in the 3xTG and 5xFAD mouse lines for the testing potential pharmaceutical agents using the 5-CSRTT in the future.

4.2 Basis of Attention Deficits in AD

Translation of data from mouse experiments to humans may also be improved if we were to expand our scope in preclinical studies to include not only memory, but also other relevant cognitive brain constructs such as attention. Individuals with AD have impaired executive control, part of which is attention (Perry and Hodges, 1999, Baddeley et al., 2001). Clinical observation of AD patients has revealed they have difficulty performing simple tasks at a relatively early stage in the disease, often described easily distractible, unable to concentrate and have difficulty with tasks that were previously easily performed (Perry and Hodges, 1999). Recent work from Dr. Bussey and Dr. Saksida's lab has shown the 3xTG male mice, older than 10 months, have deficits in attention using the 5-CSRTT on the touchscreen systems (Romberg et al., 2011). The results obtained in this study for 3xTG males reproduce those observed by Dr. Bussey's lab. Other work on the TgCRND8 mouse model of AD has shown deficits in attention during the latter stages of life (Romberg et al., 2011, Romberg et al., 2013b). Our study demonstrates a systematic deficit in 5-CSRTT performance with age in both the 3xTG and 5xFAD mouse models of AD.

Current evidence suggests that attention is the first non-memory domain to be affected in AD before deficits in visuospatial functions and language are observed (Perry and Hodges, 1999, Romberg et al., 2013a). Hence, recognizing the onset of attention deficits may help in the early diagnosis of AD, before it progresses to other cognitive domains. This is an important step in testing potential pharmacological interventions that act early and prevent the progression of AD. Different mouse models of AD have attention deficits at different ages depending on a number of factors including the types of mutations the mice carry (Webster et al., 2014). Our study demonstrated that the onset of attention deficits in the 3xTG and 5xFAD mouse models differs, with 4 and 7 months of age respectively. More importantly, this helps establish a baseline for the onset of AD-like attention deficits in these two mouse models of AD. The majority of behavioural studies using these or other mouse models of AD test cognitive functions when the mice are in later stages of life, in order to mimic the symptoms of AD seen in humans (Romberg et al., 2011, Romberg et al., 2013b). Unfortunately, not only does this increase the costs of maintaining mice as they age, but it also decreases the success of potential therapeutics as the AD-like pathology may be too far advanced in the older mice.

4.3 3xTG Mice Show Attention Deficits Earlier than 5xFAD

We show that both the 3xTG and 5xFAD mouse lines exhibit attention deficits using the 5-CSRTT. The 3xTG male and female mice showed deficits in accuracy, the main measure of attention in this task, at 4.5 months of age and throughout the other two time points at 7 months and 10 months. On the other hand, the 5xFAD male and female mice showed deficits in accuracy starting at 7 months and more statistically significant deficits

at 10 months, but showed no difference at 4.5 months. This difference in performance could be a result of the additional tau mutation the 3xTG mice carry. The 5xFAD mice have three mutations on the APP gene (APP^{SWE}, APP^{Fl}, APP^{LON}) as well as two mutations on the PSEN1 gene (M146L and L286V) (Oakley et al., 2006). The 3xTG mice on the other hand have one mutation for APP (APP^{SWE}), one on PSEN1 (M146L) and a mutation on the tau transgene (P301L) (Oddo et al., 2003b). The 3xTG mice show accumulation of both plaques and tangles in AD relevant brain regions as well as loss in synaptic plasticity and cholinergic receptors (Billings et al., 2005). In the 3xTG mice, the first detectable pathology is the development of intracellular A β at 3 months followed by detection of both extracellular A β plaques and hyperphosphorylated tau in the hippocampus at 6 months (Billings et al., 2005, Mastrangelo and Bowers, 2008). The 5xFAD mice however, have been shown to have intracellular A β detectable at 1.5 months and extracellular plaques at 2 months (Oakley et al., 2006).

Although many studies have suggested that A β is the initiating factor of AD and tau dysfunction is a downstream factor to A β aggregation (Hardy and Allsop, 1991, Hardy and Selkoe, 2002) other studies have suggested that tau interacts with A β and accelerates the progression of AD (Oddo et al., 2006a). Numerous studies have also shown that soluble oligomers are the most toxic form of A β to neurons (Harper et al., 1997, Walsh and Selkoe, 2007) and several others have shown that soluble tau oligomers may also be more toxic in AD (Berger et al., 2007, Roberson et al., 2007, Lasagna-Reeves et al., 2011, Castillo-Carranza et al., 2014). More recent studies have also revealed that soluble tau species including prefibrillar, oligomeric and prefilament forms are more toxic in AD than the hallmark NFTs, which may in fact have a more protective role (Bretteville and

Planel, 2008, Cowan and Mudher, 2013). Hence, in the 3xTG mice, oligomeric tau protein may be leading to the early deficits in attention observed in the 5-CSRTT.

The AD-like attention deficits observed in both the male and female 5xFAD mice at 7 months and onwards can be attributed to the mutations in APP and PSEN-1 that lead to A β plaques. Attentional control, part of executive functions, is controlled by a myriad of cortical and subcortical regions. The 5xFAD mice have been shown to have A β accumulation in cortical regions by 6 months of age, followed by synaptic degeneration and loss of neurons by 9 months (Oakley et al., 2006, Bilkei-Gorzo, 2014). This coincides with the deficits in attention we observed in the 5xFAD mice, first appearing at 7 months and deteriorating significantly at 10 months, for both sexes. Thus, the deficits in attention observed in the 5xFAD mouse line suggests they are a direct result of A β accumulating in the cortex.

4.4 Other Cognitive deficits in AD-mouse models revealed by the 5-CSRTT

Similar to the attention deficits observed, the 3xTG mice also showed very significant deficits in correct response latency throughout all three probe trials. Correct response latency is the amount of time a mouse takes to touch the screen after the onset of a stimulus. This is a measure of cognitive processing speed (Romberg et al., 2011) and the 3xTG mice took significantly longer from the first time point of 4.5 months and onwards. The 5xFAD mice also showed deficits in correct response latency but these were only present during the later time points. The cognitive processing of the 3xTG mice so early

in life can be again attributed to the presence of the tau (P301L) mutation. Recent studies have shown that levels of hyperphosphorylated tau protein, NFTs, but not A β load, correlates strongly with cognition in AD (Giacobini and Gold, 2013). In one study, APP mice that showed cognitive deficits were crossed with tau knockout mice to make a new mouse model that lower levels of tau. This had no effect on the development of A β pathology; however, it did prevent the cognitive deficits that were seen originally in the APP mice (Roberson et al., 2007). Findings in studies using transgenic animals carrying tau mutations also seem more closely aligned with human AD studies (LaFerla and Green, 2012). The 5xFAD mice also showed deficits in correct response latencies but during the last probe trials. Evidence from previous research has suggested that A β formation can drive tau pathology in patients with AD (Handoko et al., 2013). Transgenic mice that overexpress mutated forms of APP, like the 5xFAD mice, are the most commonly used animal models of AD in research. Despite extensive A β accumulation, these mice do not develop NFTs, but research has shown that they can exhibit elevated levels of hyperphosphorylated tau (Oddo et al., 2006b). This may be occurring in the 5xFAD mice as well, in the later stages of their life, after extensive A β plaque accumulation has occurred leading to the deficits in cognitive processing speed that we observed during the 10 months time point.

On the other hand, the 5xFAD mice also demonstrated significant differences in preservative correct and reward collection latency during the 10 months probe trial. Although food restricted, these mice showed a lack of motivation (higher reward collection latency) and were more placid (decreased preservative correct) compared to their age-matched wild-type littermates. These deficits at 10 months, along with the

deficits in accuracy and correct response latency, may be occurring due to the widespread A β accumulation that leads to neurodegeneration in these mice starting at 9 months of age (Oakley et al., 2006). As previously shown, the 5xFAD mice have extensive A β deposition in the cortex, subiculum, and hippocampus from 9 months onwards (Oakley et al., 2006). At 9 months of age, 5xFAD transgenic mice have only 75% of the neurons that age-matched controls possess due to synaptic degeneration (Oakley et al., 2006). Hence, this may be the driving factor to the cognitive deficits observed in this mouse line.

4.5 Male 3xTG and 5xFAD mice show increased attention deficits with age compared to females

The male 3xTG and 5xFAD mice showed greater deficits in attention with age compared to females. At the last time point of 10 months, the males from both sites demonstrated not only deficits in accuracy, but also in omissions. The females on the other hand did not show any difference in omission. Although accuracy is the main measure of attention in the 5-CSRTT, differences in omission can also represent deficits in attention as it determines whether there is a complete failure to pay attention the stimulus displayed on the screen (Romberg et al., 2013a). Thus, the males from both mouse lines seem to have greater AD-like cognitive deficits in attention at this age than females.

Research has suggested that estrogen, the female sex hormone, can play a protective role in the brain of females against AD, and loss of estrogen during menopause leads to deficits in brain metabolism leading to the hallmark lesions seen in the disease (Long et al., 2012). Estrogen has also been shown to have a critical role in neurogenesis in various

regions including the hippocampus and other subcortical regions leading to the generation of neurons that contribute to region-specific learning and memory (Galea et al., 2013). Several studies have provided insight into the importance of estrogen on neuronal morphology and synaptic plasticity in the CA1 region of the hippocampus (Mukai et al., 2010, Hojo et al., 2011, Ooishi et al., 2012, Spencer-Segal et al., 2012). Moreover, estrogen reduces levels of A β in the presence of pathological triggers (Anastasio, 2013). Research has also shown that estrogen can help reduce levels of A β production by decreasing BACE1 enzyme levels, and increasing A β clearance by stimulation of microglia degradation and phagocytosis as well as increasing levels of other enzymes that are involved in A β degradation (Singh et al., 1999). Additionally, studies have also shown that estrogen has a role in decreasing levels of hyperphosphorylated tau by activating kinases and phosphatases such as the Wnt, GSK-3 β and PKA pathways (Zhang et al., 2008). The loss in estrogen during menopause in females has been suggested to account for the increased susceptibility to AD compared to males (Yue et al., 2005, Li et al., 2013). Furthermore, one study demonstrated decreased levels of estrogen in the CSF of AD patients providing additional confirmation of its role in the disease (Schonknecht et al., 2001).

Moreover, in a recent study that used aged female 3xTG mice, there was an increase in AD pathology in transgenic mice who had an ovariectomy, leading to reduced levels of estrogen and other gonadotropins, compared to age-matched transgenic females that did not (Palm et al., 2014). In addition, research shows that levels of estrogen and testosterone decrease steadily with age in both males and pre-menopausal females (Ferrini and Barrett-Connor, 1998). Thus, the attention deficits in the mice at 10 months

may also be due to a slight decrease in levels of estrogen with age, leading to an increase in AD-like pathology. Overall, our study suggests estrogen may be playing a protective role in both the 3xTG and 5xFAD female mice. The lower levels of estrogen in males may be contributing to greater AD-like pathology thus leading to greater attention deficits with age.

4.6 Conclusion

In summary, we provide a good baseline for the onset and progression of AD-like attention deficits in both the 3xTG and 5xFAD mouse lines. Due to their three mutations that lead to both A β plaques as well as tau NFTs, both the male and female 3xTG mice showed deficits in attention during the first time point and onwards of this longitudinal study. The 5xFAD males and females on the other hand, with their five additive mutations that lead an accelerated rate of amyloidosis, showed deficits from the 7 months time point and onwards. In addition, the majority of data was reproduced between site 1 and site 2, attesting to the reliability of the touchscreen systems used in this study. Transgenic male mice from both lines showed greater deficits in attention with age compared to female mice.

4.7 Future Directions

Further behavioural testing will be done using both the 3xTG and 5xFAD mice on the touchscreen systems to determine if and when they have deficits in other cognitive domains. The PAL task will be utilized to test for spatial memory deficits and the PD task

will be used to evaluate whether the mice have deficits in extinction learning. In addition, another mouse model of AD, the APP/PSEN1 male and female mice will be used on all three touchscreen tasks (5-CSRTT, PAL, and PD) to determine whether they also display AD-like cognitive symptoms. Furthermore, we plan on imaging the brains of all three mouse lines at the three probe trial time points used in the 5-CSRTT in order to determine the extent of AD-like plaques and tangles these mice display. In the near future, we also plan on testing potential pharmacological agents that may help treat the cognitive symptoms associated with AD using the touchscreen systems and the baselines established in order to accelerate this process.

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Appendix A

TITLE: 5-Choice Serial Reaction Time Task (5-CSRT)

SOP NO.:

DATE:

1.0 INTRODUCTION

The 5-CSRT task has been designed to measure effects of drugs and other manipulations (ex: genetic) on attentional performance (and stimulus control). The test is performed in a specially designed touchscreen-based automated chamber with multiple response locations ('five-windows') using food reinforcers to maintain performance. The 5CSRTT is useful for measuring effects of different manipulations on various aspects of attentional control, including sustained, selective and divided attention – and is relevant to the definition of neural systems of attention and has applications to human disorders such as attention deficit/hyperactivity disorder (ADHD) and Alzheimer's disease.

2.0 EQUIPMENT

- Mouse Touch Screen Systems and ABET II
- 89543CAM 5-Choice Serial Reaction Time Task with Cambridge Amendment
-

3.0 PROCEDURE

3.1 Testing the hardware:

A quick test of the hardware should be done prior to every days training or testing. To do the hardware testing, follow procedures indicted below:

- a. From the main menu the Execution Manager, select the boxes you wish to test.
- b. Click the 'Open/Load Schedule' icon and select 'Touch MouseTestLines' and click 'Open'.
- c. Click the play icon. The boxes are now ready to test.

See Table 1 for Inputs to activate a response and output response expected.

Table 1: Action necessary and output response expected.

Schedule	Inputs to activate (use your fingers)	Output response
Touch Mouse Test Lines	Touch Grid 1	Image in all grid spaces, Grid 1 has 30% of full white, grids 2 to 4 have full white image. <i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 2	Image in all grid spaces, Grid 2 has 40% of full white, all other grids have full white image. <i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 3	Image in all grid spaces, Grid 3 has 50% of full white, all other grids have full white image.

		<i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 4	<p>Image in all grid spaces, Grid 4 has 70%</p> <p>of full white, all other grids have full white image.</p> <p><i>Activate the Tray to clear all images and proceed with test.</i></p>
	Touch Grid 5	<p>Full white (bright) image in all grid spaces. Pulses Sound_On 500ms</p> <p><i>Activate the Tray to clear all images and proceed with test.</i></p>
	Enter the feed-tray	House-light and tray-light illuminate and feeder - 800ms
	Exit the feed-tray	Houselight and tray light extinguish
	Block Front IR activity beams	House-light illuminates
	Block Back IR activity beams	Tray-light illuminates

3.2 Testing the feeder and mask

- A quick test of the feeder should be done prior to every days training or testing. That is, turn on manually the switch on the feeder pump and make sure the food is delivered.
- Make sure the 5CSRT Mask is inserted (5 windows).
- Reward provided is Neilson Strawberry milkshake (SM) (Saputo Inc. Montreal Quebec. H1P1X8). This milkshake can be found in most grocery stores (including Wall Mart and Superstore).

3.3 Pre-training

- Animals need to be food restricted before task training and throughout experiment (see relevant SOPs: “Food restriction in young mice” or “Food restriction in adult mice”).
- Divide the subjects of each group to be tested (Ex: Group 1: 5xFAD females, Group 2: APP males) into 4 sub-groups (A, B, C, D). Groups must be counter-balanced for genotype (wt x mutant). Each subgroup is going to follow specific testing schedules during probe trial (see Table 2).

3.4 Training Procedures

3.4.1 Basic training schedule

IMPORTANT: for both training and probe trials, each mouse is submitted to one session per day.

Stage 1: Habituation1: 1 session. Load the habituation1 schedule from the CAM-5choice subdirectory in the ABETII software. The session duration is set to 600s (10 minutes), and the number of trials is left to unlimited. Mouse is left in the chamber for 10 min. All lights are turned off. No stimulus or reward is presented. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 2: Habituation2a: 2 sessions. Load the habituation2 schedule from the CAM-5choice subdirectory in the ABETII software. The session duration is set to 1200s (20 minutes), and the number of trials is left to unlimited. The mouse is left in the chamber for 20 min sessions. The tray light is going to be initially turned on. A tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivered for 6000ms (150µl). The program waits for the mouse to enter the food tray. When the mouse leaves the reward tray, the reward tray light is turned off. There is a 10s delay before the tray light is turned on, a tone is played and SM is then delivered for 280ms (7 µl). If the

mouse is in the reward tray at the end of the 10s delay, an extra 1s is added to the delay. The procedure is repeated until the session ends.

Stage 3: Habituation2b: 1 session. Load the habituation2 schedule from the CAM-5choice subdirectory in the ABETII software. The session duration is set to 2400s (40 minutes), and the number of trials is left to unlimited. The mouse is left in the chamber for 40 min. Reward presentation is the same as described in stage 2. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 4: “Initial touch”: (usually 1 session). Load the schedule ‘5-choice Mouse Initial Touch Training’ from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure that “Image Time” is 30s; ‘Feed Pulse Time’ is 280ms; “tone duration” is 1000 ms, and ITI period is 5s. The stimulus (a white square) is displayed randomly in one of the 5 windows. The stimulus is presented in only one window at a time. The other windows are left blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. After a delay (**Image Time – 30s**) the image is removed and food is delivered (**‘Feed Pulse Time –280ms**). Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency is 3 KHz. The tone duration is (**1000 ms**). Entry to collect the food turns off the tray light and starts the ITI. After the **ITI period (5s)** another image is displayed. If the mouse touches the screen whilst the image is displayed (where the image is displayed), the image is removed, a tone will be played and 3 x food is delivered immediately. Collection of this reward again starts the ITI and then progresses to the next image. Touch training is performed with the house light off.

Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

Stage 5: “Must touch”: Number of session varies for individual mouse. It can go from ~1-7 days (median: 2 days). Run the schedule ‘5-choice Mouse Must Touch Training’ from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No Trials to 30. Make sure **tone duration is set to 1000 ms** (from the ‘Tone Duration’ variable) and **ITI period is set to 5s**.

The stimulus (a white square) is displayed randomly in one of the 5 windows. The stimulus is presented in only one window at a time. The other windows are left blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency

default is 3 KHz. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (5s) another image is displayed.

Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

IMPORTANT: If after 7 sessions a mouse does not reach criterion for “must touch”, take it back one step; that is, retrain the mouse on “Initial touch” until it reaches criterion and repeat the “Must touch” training. If after 7 sessions of the second attempt of “must touch” the mouse does not reach criterion, remove it from the study.

Stage 6: “Must initiate”: Number of session varies for individual mouse. It usually takes ~1-2 sessions (i.e. 1 or days). Run the schedule ‘5-Choice Must Initiate Training’ from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure **tone duration is set to 1000 ms** (from the ‘Tone Duration’ variable) and **ITI period is set to 5s**.

A free delivery of food is made and the tray light is turned on. The mouse must nose poke and exit the reward tray before a stimulus is displayed randomly on the screen. The stimulus (a white square) is displayed randomly in one of the 5 windows. The stimulus is presented in only one window at a time. The other windows are left blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row.) The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches the blank parts of the screen. Food delivery is accompanied by illumination of the tray light and a tone. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period the tray light is again illuminated. The mouse must nose poke and exit the reward tray before the next image is displayed.

Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

IMPORTANT: If after 5 sessions a mouse does not reach criterion for “must initiate”, take it back one step; that is, retrain the mouse on “must touch” until it reaches criterion and repeat the “Must Initiate” training. If after 5 sessions of the second attempt of “must initiate” the mouse does not reach criterion, remove it from the study.

Stage 7: “Punish incorrect”. Number of session varies for individual mouse. It can go from ~2-30 days (median: 9 days). Run the schedule ‘5-Choice Mouse Punish Incorrect Training’ from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure **tone duration is set to 1000 ms** and **ITI period is set to 5s**.

This schedule trains the mouse to both initiate after an ITI and not to touch an incorrect location. As for previous training described above, except if a mouse touches an incorrect (non-illuminated) location the **house light will be turned ON for 5s** and **no reward is given**. Once the time out period finishes the house light is turned OFF again and the **ITI period begins (5s)**. The mouse must then complete a correction trial: the image and position from the previous trial are kept the same and the mouse must repeat the same trial until a correct response to the image is made, at which point it will receive a tone and reward.

Criterion: Completion of 23/30 trials or better within 60 min for 2 consecutive sessions

IMPORTANT: If after 30 sessions (30 days) the mouse does not reach criterion for “Punish incorrect”, remove it from study.

3.4.2 5-CSRT Training to baseline

Stage 8: 5-CSRT training to baseline- 4s stimulus. Number of session varies for individual mouse. It can go from ~4-30 days (median: 11 days). Run the ‘5CSRTT_4s_Var1’ from the CAM-5choice subdirectory schedule. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50. Make sure tone duration is set to 1000 ms, ITI period is set to 5s, Food/CM pulse time [280ms (7 μ l SM), Delay interval (5s), time out (TO, 5s) and ITI Incorr (5s).

*The session begins with a priming delivery of reinforcer [280ms (7 μ l SM)] and on exiting the food magazine the first trial begins. Following tray exit, a “Delay interval” (5s) begins at the end of which a stimuli is presented in one of the 5 stimuli grid spaces on the LCD touch screen. The sequence of presentations of the stimuli is a pseudorandom schedule such that there are 4 presentations at each spatial location within a block of 20 trials. The subject must respond within a time period defined (**limited hold period 5s**). A correct response, touching at the location in which the stimulus was presented, will trigger the presentation of reinforcer [280ms (7 μ l SM)] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is (**1000 ms tone**). The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (5s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial and start the Delay’ interval. An incorrect response, i.e. touching a location other than where the stimulus was presented, or making no response at all (an omission) within the limited hold period, will cause a time out (**TO, 5s**) as identified house light turned ON. After the TO, the house light will be turned OFF and the “**ITI Incorr**” will begin (**5s**). After the ITI incorr period the tray light will come on and the subject must enter and exit the food tray to start the next trial and start the Delay’ interval. A premature response is recorded when a touch is made in one of the response grid areas during the Delay and also results in a TO.*

> 80% accuracy = [number of Correct trials / Total number of trials responded to (correct and incorrect)]

< 20% omissions = [number of trials missed / number of trials presented]

Criterion: 80% accuracy or better, 20% omission or less, 3 consecutive days, minimum 30 trials completed per session.

Stage 9: 5-CSRT training to baseline- 2s stimulus. Number of sessions varies for individual mouse. It can go from ~5-30 days (median: 12 days). Run the '5CSRTT_2s_Var1' schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50. . Make sure tone duration is set to 1000 ms, ITI period is set to 5s, Food/CM pulse time [280ms (7 μ l SM), Delay interval (5s), time out (TO, 5s) and ITI Incorr (5s).

The session begins with a priming delivery of reinforce [280ms (7 μ l SM)] and on exiting the food magazine the first trial begins. Following tray exit, a “**Delay interval**” (5s) begins at the end of which a stimuli is presented in one of the 5 stimuli grid spaces on the LCD touch screen. The sequence of presentations of the stimuli is a pseudorandom schedule such that there are 4 presentations at each spatial location within a block of 20 trials. The subject must respond within a time period defined (**limited hold period 5s**). A correct response, touching at the location in which the stimulus was presented, will trigger the presentation of reinforce [280ms (7 μ l SM)] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is (**1000 ms tone**). The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (5s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial and start the Delay' interval. An incorrect response, i.e. touching a location other than where the stimulus was presented, or making no response at all (an omission) within the limited hold period, will cause a time out (**TO, 5s**) as identified house light turned ON. After the TO, the house light will be turned OFF and the '**ITI Incorr**' will begin (**5s**). After the ITI incorr period the tray light will come on and the subject must enter and exit the food tray to start the next trial and start the Delay' interval. A premature response is recorded when a touch is made in one of the response grid areas during the Delay and also results in a TO.

Criterion: 80% accuracy or better, 20% omission or less, 3 consecutive days, 50 trials must be completed per session.

3.4.3 Testing schedules

Stage 10: First probe trial evaluation. Subjects will not progress through the training at exactly the same rate. The first set of probe trials for a group begins once the last mouse in that group has passed the 2s stimulus performance criteria (Stage 9). Subjects that have completed the Stage 9 before the slowest subject are maintained on food restriction and repeat Stage 9 before performing their probe trial. There is no minimum performance criterion for subjects to advance through the probe trials. The order of performance of probe trials for each counter-balanced group varies according to Table 2.

- For the 2s stimulus run the '5CSRTT_2s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 1.5s stimulus run the '5CSRTT_1.5s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 1s stimulus run the '5CSRTT_1s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 0.8s stimulus run the '5CSRTT_0.8s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 0.6s stimulus run the '5CSRTT_0.6s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.

Table 2: Order of stimulus duration for individual groups (1st probe trial evaluation)

# of consecutive sessions	Stimulus duration throughout sessions for Sub-group A	Stimulus duration throughout sessions for Sub-group B	Stimulus duration throughout sessions for Sub-group C	Stimulus duration throughout sessions for Sub-group D
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2	0.6s	0.8s	1.0s	1.5s
2	2.0s	2.0s	2.0s	2.0s
2	1.5s	0.6s	0.8s	1.0s
2	2.0s	2.0s	2.0s	2.0s
2	1.0s	1.5s	0.6s	0.8s
2	2.0s	2.0s	2.0s	2.0s
2	0.8s	1.0s	1.5s	0.6s

Table 3: Setting adjustment for additional variables in probe trials

Variable	Value
Session Length	60 min
Food/CM pulse time	280 ms
DELAY	5s
Time out	5s
Limited Hold Value	5s

Stage 11: Reusing same mouse cohort for a new probe trial

- All subjects are maintained on food restriction for 1 month.

- Subjects perform one 2s stimulus trial per week during the interval between probe trials.

Stage 12: Second probe trial evaluation

- Mice should be re-baselined at 2s (Stage 9: >80% Accuracy, <20% omissions). Depending on how long it's been since the previous probe trial it might be necessary to rebaseline them at 4s first (Stage 8). If they are not re-baselined the second probe trial will not be accurate.
- A second probe trial should be performed according to the order shown in Table 4.

Table 4: Order of stimulus duration for individual groups (2nd probe trial evaluation)

# of consecutive sessions	Stimulus duration throughout sessions for Sub-group A	Stimulus duration throughout sessions for Sub-group B	Stimulus duration throughout sessions for Sub-group C	Stimulus duration throughout sessions for Sub-group D
2	1.5s	0.6s	0.8s	1.0s
2	2.0s	2.0s	2.0s	2.0s
2	1.0s	1.5s	0.6s	0.8s
2	2.0s	2.0s	2.0s	2.0s
2	0.8s	1.0s	1.5s	0.6s
2	2.0s	2.0s	2.0s	2.0s
2	0.6s	0.8s	1.0s	1.5s

Curriculum Vitae

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